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#### COMPOUNDS

This application has the same text and claims as GB 9818733.9, except for new description pages 210 to 212, and new claims 41 to 43.

The present invention relates to pyrrolobenzodiazepines (PBDs).

#### Background to the invention

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Some pyrrolobenzodiazepines (PBDs) have the ability to recognise and bond to specific sequences of DNA; the preferred sequence is PuGPu. The first PBD antitumour antibiotic, anthramycin, was discovered in 1965 (Leimgruber et al., 1965 J. Am. Chem. Soc., 87, 5793-5795; Leimgruber et al., 1965 J. Am. Chem. Soc., 87, 5791-5793). Since then, a number of naturally occurring PBDs have been reported, and over 10 synthetic routes have been developed to a variety of analogues (Thurston et al., 1994 Chem. Rev. 1994, 433-465). Family members include abbeymycin (Hochlowski et al., 1987 J. Antibiotics, 40, 145-148), chicamycin (Konishi et al., 1984 J. Antibiotics, 37, 200-206), DC-81 (Japanese Patent 58-180 487; Thurston et al., 1990, Chem. Brit., 26, 767-772; Bose et al., 1992 Tetrahedron, 48, 751-758), mazethramycin (Kuminoto et al., 1980 J. Antibiotics, 33, 665-667), neothramycins A and B (Takeuchi et al., 1976 J. Antibiotics, 29, 93-96), porothramycin (Tsunakawa et al., 1988 J. Antibiotics, 41, 1366-1373), prothracarcin (Shimizu et al, 1982 J. Antibiotics, 29, 2492-2503; Langley and Thurston, 1987 J. Org. Chem., 52, 91-97), sibanomicin (DC-102) (Hara et al., 1988 J. Antibiotics, 41, 702-704; Itoh et al., 1988 J. Antibiotics, 41, 1281-1284), sibiromycin (Leber et al., 1988 J. Am. Chem. Soc., 110, 2992-2993) and tomamycin (Arima et al., 1972 J. Antibiotics, 25, 437-444). PBDs are of the general structure:

They differ in the number, type and position of substituents, in both their aromatic A rings and pyrrollo C rings, and in the degree of saturation of the C ring. In the B-ring there is either an imine (N=C), a carbinolamine(NH-CH(OH)), or a carbinolamine methyl ether (NH-CH(OMe))at the N10-C11 position which is the electrophilic centre responsible for alkylating DNA. All of the known natural products have an (S)-configuration at the chiral C11a position which provides them with a right-handed twist when viewed from the C ring towards the A ring. This gives them the appropriate three-dimensional shape for isohelicity with 10 the minor groove of B-form DNA, leading to a snug fit at the binding site (Kohn, 1975 In Antibiotics III. Springer-Verlag, New York, pp. 3-11; Hurley and Needham-VanDevanter, 1986 Acc. Chem. Res., 19, 230-237). Their ability to form an adduct in the minor groove, enables them to interfere with DNA processing, 15 hence their use as antitumour agents.

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#### Disclosure of the invention

A first aspect of the present invention is a compound with the formula Ia or Ib:

wherein:

A is  $CH_2$ , or less preferably a single bond; 5  $R_2$  is selected from: R, OH, OR,  $CO_2H$ ,  $CO_2R$ , COH, COR,  $SO_2R$ , CN;  $R_6$ ,  $R_7$  and  $R_9$  are independently selected from H, R, OH, OR, halo, amino, NHR, nitro, Me<sub>3</sub>Sn; where R is a lower alkyl group having 1 to 10 carbon atoms, or an aralkyl group (i.e. an alkyl group with one or more aryl 10 substituents), preferably of up to 12 carbon atoms, whereof the alkyl group optionally contains one or more carbon-carbon double or triple bonds, which may form part of a conjugated system, or an aryl group, preferably of up to 12 carbon atoms; and is optionally substituted by one or more halo, hydroxy, 15 amino, or nitro groups, and optionally containing one or more hetero atoms which may form part of, or be, a functional group; and R<sub>8</sub> is selected from H, R, OH, OR, halo, amino, NHR, nitro, Me<sub>3</sub>Sn, where R is as defined above, or the compound is a dimer with each monomer being the same or different and being of 20 formula Ia or Ib, where the R<sub>8</sub> groups of the monomers form together a bridge having the formula -X-R'-X- linking the

monomers, where R' is an alkylene chain containing from 3 to 12 carbon atoms, which chain may be interrupted by one or more hetero-atoms and/or aromatic rings, e.g. benzene or pyridine, and may contain one or more carbon-carbon double or triple bonds, and each X is independently selected from O, S, or N; except that in a compound of formula  $\mathbf{Ia}$  when A is a single bond, then  $\mathbf{R}_2$  is not  $\mathbf{CH}=\mathbf{CH}(\mathbf{CONH}_2)$  or  $\mathbf{CH}=\mathbf{CH}(\mathbf{CONMe}_2)$ .

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If A is a single bond then  $R_2$  is bonded directly to the C-ring of the PBD.

- 10 If R is an aryl group, and contains a hetero atom, then R is a heterocyclic group. If R is an alkyl chain, and contains a hetero atom, the hetero atom may be located anywhere in the alkyl chain, e.g. -O-C<sub>2</sub>H<sub>5</sub>, -CH<sub>2</sub>-S-CH<sub>3</sub>, or may form part of or be a functional group e.g. carbonyl, hydroxy.
- It is preferred that in a compound of formula  ${\bf Ia}$  when A is a single bond, then  $R_2$  is not  ${\bf CH=CR^AR^B}$ , where  ${\bf R^A}$  and  ${\bf R^B}$  are independently selected from H,  ${\bf R^C}$ ,  ${\bf COR^C}$ ,  ${\bf CONH_2}$ ,  ${\bf CONHR^C}$ ,  ${\bf CONR^C}_2$ , cyano or phosphonate, where  ${\bf R^C}$  is an unsubstituted alkyl group having 1 to 4 carbon atoms.
- 20 R is preferably selected from a lower alkyl group having 1 to 10 carbon atoms, or an aralkyl group, preferably of up to 12 carbon atoms, or an aryl group, preferably of up to 12 carbon atoms, optionally substituted by one or more halo, hydroxy, amino, or nitro groups. It is more preferred that R is selected from a

lower alkyl group having 1 to 10 carbon atoms optionally substituted by one or more halo, hydroxy, amino, or nitro groups. It is particularly preferred that R is an unsubstituted straight or branched chain alkyl, having 1 to 10, preferably 1 to 6, and more preferably 1 to 4, carbon atoms, e.g. methyl, ethyl, n-propyl, n-butyl or t-butyl.

Alternatively,  $R_6$ ,  $R_7$ ,  $R_9$  and, unless the compound is a dimer,  $R_8$  may preferably be independently selected from R groups with the following structural characteristics:

10 (i) an optionally substituted phenyl group;

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- (ii) an optionally substituted ethenyl group;
- (iii) an ethenyl group conjugated to an electron sink.

The term 'electron sink' means a moiety covalently attached to a compound which is capable of reducing electron density in other

parts of the compound. Examples of electron sinks include cyano, carbonyl and ester groups.

It is preferred that A is  $CH_2$  and/or that  $R_2$  is  $CO_2H$ ,  $CO_2R$ ,  $CH_2OH$ , or  $CH_2OR$ . It is further preferred that  $R_2$  is  $CO_2Me$ ,  $CO_2^{\dagger}Bu$ ,  $CH_2OH$ , or  $CH_2OAc$ .

 $R_6$ ,  $R_7$ , and  $R_9$ , unless the compound is a dimer,  $R_8$  are preferably selected from H and OR, and more particularly H, OMe and OCH<sub>2</sub>Ph. It is further preferred that  $R_7$  and, unless the compound is a dimer,  $R_8$  are OR, more preferably OMe or OCH<sub>2</sub>Ph, and that  $R_6$  and  $R_9$  are H.

Compounds of the first aspect of the invention are preferably of formula Ia.

If the compound of formula  ${\bf Ia}$  or  ${\bf Ib}$  is a dimer, the dimer bridge may be of the formula  $-O-(CH_2)_p-O-$ , where p is from 1 to 12, more preferably 3 to 9.

A second aspect of the present invention is a compound with the formula II:

wherein:

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 ${\rm R'}_2$  is selected from: O, CHR", where  ${\rm R"}_2$  is selected from H, R,  ${\rm CO}_2{\rm R}$ , COR, CHO,  ${\rm CO}_2{\rm H}$ , halo;

 $R_6$ ,  $R_7$  and  $R_9$  are independently selected from H, R, OH, OR, halo, amino, NHR, nitro, Me<sub>3</sub>Sn;

where R is a lower alkyl group having 1 to 10 carbon atoms, or an aralkyl group (i.e. an alkyl group with one or more aryl substituents), preferably of up to 12 carbon atoms, whereof the alkyl group optionally contains one or more carbon-carbon double

or triple bonds, which may form part of a conjugated system, or an aryl group, preferably of up to 12 carbon atoms; and is optionally substituted by one or more halo, hydroxy, amino, or nitro groups, and optionally containing one or more hetero atoms which may form part of, or be, a functional group; and  $R_8$  is selected from H, R, OH, OR, halo, amino, NHR, nitro,  $Me_3Sn$ , where R is as defined above or the compound is a dimer with each monomer being the same or different and being of formula II, where the  $R_8$  groups of the monomers form together a bridge having the formula -X-R'-X- linking the monomers, where R' is an alkylene chain containing from 3 to 12 carbon atoms, which chain may be interrupted by one or more hetero-atoms and/or aromatic rings, e.g. benzene or pyridine, and may contain one or more carbon-carbon double or triple bonds, and each X is independently selected from O, S, or N; except that:

- (i) when  ${\rm R'}_2$  is CH-Et, and  ${\rm R}_6$ ,  ${\rm R}_8$  and  ${\rm R}_9$  are H,  ${\rm R}_7$  is not sibirosamine pyranoside; and
- (ii) when  ${\rm R'}_2$  is CH-Me, and  ${\rm R}_6$  and  ${\rm R}_9$  are H,  ${\rm R}_7$  and  ${\rm R}_8$  are not both H or both OMe, or OMe and OH respectively.

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If R is an aryl group, and contains a hetero atom, then R is a heterocyclic group. If R is an alkyl chain, and contains a hetero atom, the hetero atom may be located anywhere in the alkyl chain, e.g.  $-O-C_2H_5$ ,  $-CH_2-S-CH_3$ , or may form part of or be a functional group e.g. carbonyl, hydroxy.

R is preferably selected from a lower alkyl group having 1 to 10 carbon atoms, or an aralkyl group, preferably of up to 12 carbon atoms, or an aryl group, preferably of up to 12 carbon atoms, optionally substituted by one or more halo, hydroxy, amino, or nitro groups. It is more preferred that R is selected from a lower alkyl group having 1 to 10 carbon atoms optionally

substituted by one or more halo, hydroxy, amino, or nitro groups. It is particularly preferred that R is an unsubstituted straight or branched chain alkyl, having 1 to 10, preferably 1 to 6, and more preferably 1 to 4, carbon atoms, e.g. methyl, ethyl, n-propyl, n-butyl or t-butyl.

Alternatively,  $R_6$ ,  $R_7$  and  $R_9$  and, unless the compound is a dimer,  $R_8$  may preferably be independently selected from R groups with the following structural characteristics:

- (i) an optionally substituted phenyl group;
- 10 (ii) an optionally substituted ethenyl group;

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(iii) an ethenyl group conjugated to an electron sink.

It is preferred that R'2 is O, CH2 or CHCH3.

 $R_6$ ,  $R_7$ , and  $R_9$  and, unless the compound is a dimer,  $R_8$  are preferably selected from H and OR and a halogen atom, and more particularly H, OMe and OCH<sub>2</sub>Ph, and I. It is further preferred that  $R_7$  and, unless the compound is a dimer,  $R_8$  are OR or a halogen atom, more preferably OMe, OCH<sub>2</sub>Ph or I, and that  $R_6$  and  $R_9$  are H.

If the compound of formula II is a dimer, the dimer bridge may be of the formula  $-O-(CH_2)_p-O-$ , where p is from 1 to 12, more preferably 3 to 9.

A third aspect of the present invention is a compound with the formula III:

$$\begin{array}{c|c}
R_{0} \\
R_{7} \\
R_{1} \\
\end{array}$$
(III)

wherein:

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 $R_6$ ,  $R_7$  and  $R_9$  are independently selected from H, R, OH, OR, halo, amino, NHR, nitro, Me $_3$ Sn;

where R is a lower alkyl group having 1 to 10 carbon atoms, or an aralkyl group (i.e. an alkyl group with one or more aryl substituents), preferably of up to 12 carbon atoms, whereof the alkyl group optionally contains one or more carbon-carbon double or triple bonds, which may form part of a conjugated system, or an aryl group, preferably of up to 12 carbon atoms; and is optionally substituted by one or more halo, hydroxy, amino, or nitro groups, and optionally containing one or more hetero atoms which may form part of, or be, a functional group; and  $R_8$  is selected from H, R, OH, OR, halo, amino, NHR, nitro, Me<sub>3</sub>Sn, where R is as defined above or the compound is a dimer with each monomer being the same or different and being of formula III, where the  $R_8$  groups of the monomer form together a bridge having the formula -X-R'-X- linking the monomers, where R'is an alkylene chain containing from 3 to 12 carbon atoms, which chain may be interrupted by one or more hetero-atoms and/or aromatic rings, e.g. benzene or pyridine, and may contain one or more carbon-carbon double or triple bonds, and each X is independently selected from O, S, or N; wherein at least one of  $R_6$ ,  $R_7$ ,  $R_8$  and  $R_9$  are not H; except that:

- (i) when  $R_6$  and  $R_9$  are H,  $R_7$  and  $R_8$  are not both OMe, OMe and OBn respectively, or OMe and OH respectively;
- (ii) when  $R_6$  and  $R_7$  are H,  $R_8$  and  $R_9$  are not Me and OH respectively;
- 5 (iii) when three of  $R_6$ ,  $R_7$ ,  $R_8$  and  $R_9$  are H, the other is not Me;
  - (iv) when  $R_6$ ,  $R_7$ , and  $R_8$  are H,  $R_9$  is not OMe;
  - (v) when  $R_6$ ,  $R_8$  and  $R_9$  are H,  $R_7$  is not OMe; and
- (vi) when  $R_6$ , and  $R_9$  are H and  $R_7$  is OMe, the compound is not a dimer.

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If R is an aryl group, and contains a hetero atom, then R is a heterocyclic group. If R is an alkyl chain, and contains a hetero atom, the hetero atom may be located anywhere in the alkyl chain, e.g.  $-O-C_2H_5$ ,  $-CH_2-S-CH_3$ , or may form part of or be a functional group e.g. carbonyl, hydroxy.

R is preferably selected from a lower alkyl group having 1 to 10 carbon atoms, or an aralkyl group, preferably of up to 12 carbon atoms, or an aryl group, preferably of up to 12 carbon atoms, optionally substituted by one or more halo, hydroxy, amino, or nitro groups. It is more preferred that R is selected from a lower alkyl group having 1 to 10 carbon atoms optionally substituted by one or more halo, hydroxy, amino, or nitro groups. It is particularly preferred that R is an unsubstituted straight or branched chain alkyl, having 1 to 10, preferably 1 to 6, and more preferably 1 to 4, carbon atoms, e.g. methyl, ethyl, n-propyl, n-butyl or t-butyl.

Alternatively,  $R_6$ ,  $R_7$  and  $R_9$  and, unless the compound is a dimer,  $R_8$ , may preferably be independently selected from R groups with the following structural characteristics:

- (i) an optionally substituted phenyl group;
- 5 (ii) an optionally substituted ethenyl group;
  - (iii) an ethenyl group conjugated to an electron sink.

#### It is preferred that either:

- (i) only one of  $R_6$ ,  $R_7$ ,  $R_8$  and  $R_9$  is H; or
- (ii) at least one of  $R_6$ ,  $R_7$ ,  $R_8$ , and  $R_9$  is  $NH_2$ ; or
- 10 (iii) at least one of  $R_6$ ,  $R_7$ ,  $R_8$  and  $R_9$  is an aryl group, preferably of up to 12 carbon atoms, which is optionally substituted by one or more halo, hydroxy, amino, or nitro groups, and optionally contains one or more hetero atoms which may form part of, or be, a functional group.
- If only one of  $R_6$ ,  $R_7$ ,  $R_8$  and  $R_9$ , it is further preferred that the A-ring substituents (i.e. those of  $R_6$ ,  $R_7$ ,  $R_9$  and, unless the compound is a dimer,  $R_8$  which are not H) are OR, and are more preferably selected from OMe, and OBn.
- If at least one of R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub> and R<sub>9</sub> is an aryl group, preferably of up to 12 carbon atoms, which is optionally substituted by one or more halo, hydroxy, amino, or nitro groups, and optionally contains one or more hetero atoms which may form part of, or be, a functional group, it is further preferred that at least one of R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub> and R<sub>9</sub>, is a phenyl group optionally substituted by one or more methoxy, ethoxy nor nitro groups. More preferably,

the aryl group is selected from: Ph, p-MeO-Ph, m-NO<sub>2</sub>-Ph and p-NO<sub>2</sub>-Ph.

If the compound of formula III is a dimer, the dimer bridge may be of the formula  $-O-(CH_2)_p-O-$ , where p is from 1 to 12, more preferably 3 to 9.

A fourth aspect of the present invention provides a compound with the formula IV:

wherein:

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 $R_6$ ,  $R_7$  and  $R_9$  are independently selected from H, R, OH, OR, halo, amino, NHR, nitro, Me<sub>3</sub>Sn;

where R is a lower alkyl group having 1 to 10 carbon atoms, or an aralkyl group (i.e. an alkyl group with one or more aryl substituents), preferably of up to 12 carbon atoms, whereof the alkyl group optionally contains one or more carbon-carbon double or triple bonds, which may form part of a conjugated system, or an aryl group, preferably of up to 12 carbon atoms; and is optionally substituted by one or more halo, hydroxy, amino, or nitro groups, and optionally containing one or more hetero atoms which may form part of, or be, a functional group;

20  $R_8$ ' and  $R_8$ " are either independently selected from H, R or

together form a cyclic amine; and n is from 1 to 7.

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If  $R_8$ ' and  $R_8$ " form a cyclic amine, then there is usually a single N atom in a ring which is otherwise carbocyclic and is preferably 5- or 6- membered and may be saturated or unsaturated. The ring may be fused to another ring system which may be aromatic, e.g. being a benzene ring. Thus for example the cyclic amine may be an indolyl or isoindolyl group. It is also possible that the cyclic amine contains one or more hetero atoms, in addition to N in the amine ring and/or in a fused ring and may also be substituted by one or more R groups.

If R is an aryl group, and contains a hetero atom, then R is a heterocyclic group. If R is an alkyl chain, and contains a hetero atom, the hetero atom may be located anywhere in the alkyl chain, e.g.  $-O-C_2H_5$ ,  $-CH_2-S-CH_3$ , or may form part of or be a functional group e.g. carbonyl, hydroxy.

R is preferably selected from a lower alkyl group having 1 to 10 carbon atoms, or an aralkyl group, preferably of up to 12 carbon atoms, or an aryl group, preferably of up to 12 carbon atoms, optionally substituted by one or more halo, hydroxy, amino, or nitro groups. It is more preferred that R is selected from a lower alkyl group having 1 to 10 carbon atoms optionally substituted by one or more halo, hydroxy, amino, or nitro groups. It is particularly preferred that R is an unsubstituted straight or branched chain alkyl, having 1 to 10, preferably 1 to 6, and

more preferably 1 to 4, carbon atoms, e.g. methyl, ethyl, n-propyl, n-butyl or t-butyl.

R<sub>7</sub> is preferably an electron donating group, and is more preferably of the formula OR; particularly preferred are the groups OMe, OEt, and OBn. The term 'electron donating group' means a moiety covalently attached to a compound which is capable of increasing electron density in other parts of the compound.

In addition  $R_6$  and  $R_9$  are more preferably selected from H and OR; particularly preferred are OMe, OEt and OBn.

- 10 Alternatively,  $R_6$ ,  $R_7$  and  $R_9$  may preferably be independently selected from R groups with the following structural characteristics:
  - (i) an optionally substituted phenyl group;
  - (ii) an optionally substituted ethenyl group;
- 15 (iii) an ethenyl group conjugated to an electron sink.

n is preferably 1 to 3, and more preferably 1.

A fifth aspect of the present invention is the use of a compound as described in the first, second, third or fourth aspects of the invention in a method of therapy. Conditions which may be treated include gene-based diseases, including, for example, neoplastic diseases and Alzheimer's Disease, and bacterial, parasitic and viral infections. In accordance with this aspect of the present invention, the compounds provided may be

administered to individuals. Administration is preferably in a "therapeutically effective amount", this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage, is within the responsibility of general practitioners and other medical doctors.

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A compound may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

Pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may comprise, in addition to the active ingredient, i.e. a compound of formula Ia, Ib, II, III or IV, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, e.g. cutaneous, subcutaneous, or intravenous.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum,

animal or vegetable oils, mineral oil or synthetic oil.

Physiological saline solution, dextrose or other saccharide

solution or glycols such as ethylene glycol, propylene glycol or

polyethylene glycol may be included. A capsule may comprise a

solid carrier such a gelatin.

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For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

A sixth aspect of the present invention is a pharmaceutical composition containing a compound of any one of formulae Ia, Ib, II, III, or IV as described above, and a pharmaceutically acceptable carrier or diluent. The preparation of pharmaceutical compositions is described in relation to the fifth aspect of the invention above.

A seventh aspect of the present invention provides the use of a compound of any one of formulae Ia, Ib, II, III, or IV as described above to prepare a medicament for the treatment of a gene-based disease, preferably a proliferative disease. The compound of formula Ia, Ib, II, III, or IV may be provided

together with a pharmaceutically acceptable carrier or diluent. The compounds may be used for the selective killing of oxic and hypoxic tumour cells in methods for the treatment of cancers, for example leukemias and particularly solid cancers including colon, CNS, renal, and lung tumours, including small cell lung carcinoma, and melanomas. In particular, dimers of formula II may be used for the selective killing of lung, colon, and CNS tumours and melanomas. The compounds of formula III and IV may be used selectively against melanomas.

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A further aspect of the present invention provides the use of a compound of any one of formulae Ia, Ib, II, III, or IV as described above to prepare a medicament for the treatment of a viral, parasitic or bacterial infection. The preparation of a medicament is described in relation to the fifth aspect of the invention above.

In further aspects, the invention provides processes for preparing compounds according to the first, second, third and fourth aspects of the present invention.

Aspects of the invention will now be further described with

reference to the accompanying drawings in which:

Figures 1 to 5 are synthesis routes for compounds of formula Ia

of the present invention;

Figures 6 to 10 are synthesis routes for compounds of formula II

of the present invention;

25 Figures 11 to 20 are synthesis routes for compounds of formula

III of the present invention;

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Figure 21 is a synthesis of an intermediate in the preparation of compounds of formula IV of the present invention;

Figure 22 is a synthesis routes for compounds of formula IV of the present invention; and

Figures 23 to 26 are graphs illustrating the cytotoxicity results of examples 5 to 8 respectively.

#### Preferred General Synthetic Strategies

A key step in a preferred route to compounds of formula Ia, Ib, II, III or IV is a cyclisation to produce the B-ring, involving generation of an aldehyde (or functional equivalent thereof) at what will be the 11-position, and attack thereon by the Pro-N10-nitrogen:

In this structure, no C-ring substitution or unsaturation is shown.  $R_8$  represents  $O(CH_2)_nCH_2COR'_8$  in compounds of formula IV.  $R_{10}$  is a nitrogen protecting group, preferably with a carbamate functionality bonded to the nitrogen of the PBD. The "masked aldehyde" -CPQ may be an acetal or thioacetal, in which case the cyclisation involves unmasking. Alternatively, it may be an alcohol -CHOH, in which case the reaction involves oxidation, e.g. by means of TPAP or DMSO (Swern oxidation).

The masked aldehyde compound can be produced by condensing a corresponding 2-substituted pyrrolidine with a 2-nitrobenzoic acid:

The nitro group can then be reduced to  $-\mathrm{NH}_2$  and protected by reaction with a suitable agent, e.g. a chloroformate, which provides the removable nitrogen protecting group in the compound of formula I.

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A process involving the oxidation-cyclization procedure is illustrated in scheme 1 (an alternative type of cyclisation will be described later with reference to scheme 2).

Scheme 1

The imine/carbinolamine bond in the PBD  $(\mathbf{A})$  can be unprotected by standard methods to yield the desired compound, e.g. if  $R_{10}$  is Alloc, then the deprotection is carried using palladium to remove the N10 protecting group, followed by the elimination of water.

Exposure of the alcohol (B) (in which the Pro-N10-nitrogen is generally protected as carbamate) to tetrapropylammonium perruthenate (TPAP)/N-methylmorpholine N-oxide (NMO) over A4 sieves results in oxidation accompanied by spontaneous B-ring closure to afford the desired product. The TPAP/NMO oxidation procedure is found to be particularly convenient for small scale reactions while the use of DMSO-based oxidation methods, particularly Swern oxidation, proves superior for larger scale work (e.g. > 1 g).

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The uncyclized alcohol (B) may be prepared by the reaction of a nitrogen protection reagent of formula D, which is preferably a chloroformate or acid chloride, to a solution of the amino alcohol C, generally in solution, generally in the presence of a base such as pyridine (preferably 2 equivalents) at a moderate temperature (e.g. at 0°C). Under these conditions little or no O-acylation is usually observed.

The key amino alcohol C may be prepared by reduction of the corresponding nitro compound E, by choosing a method which will leave the rest of the molecule intact. Treatment of E with tin (II) chloride in a suitable solvent, e.g. refluxing methanol, generally affords, after the removal of the tin salts, the

desired product in high yield.

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Exposure of  ${\bf E}$  to hydrazine/Raney nickel avoids the production of tin salts and may result in a higher yield of  ${\bf C}$ , although this method is less compatible with the range of possible C and A-ring substituents. For instance, if there is C-ring unsaturation (either in the ring itself, or in  $R_2$  or  $R_3$ ), this technique may be unsuitable.

The nitro compound of formula  ${\bf E}$  may be prepared by coupling the appropriate o-nitrobenzoyl chloride to a compound of formula  ${\bf F}$ , e.g. in the presence of  $K_2CO_3$  at -25°C under a  $N_2$  atmosphere. Compounds of formula  ${\bf F}$  can be readily prepared, for example by olefination of the ketone derived from L-trans-hydroxy proline. The ketone intermediate can also be exploited by conversion to the enol triflate for use in palladium mediated coupling reactions.

The o-nitrobenzoyl chloride is synthesised from the o-nitrobenzoic acid (or alkyl ester after hydrolysis) of formula G, which itself is prepared from the vanillic acid (or alkyl ester) derivative H. Many of these are commercially available and some are disclosed in Althuis, T.H. and Hess, H.J., J. Medicinal Chem., 20(1), 146-266.

#### Alternative Cyclisation (Scheme 2)

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$$\begin{array}{c} R_{\bullet} \\ R_{\uparrow} \\ R_{\bullet} \\$$

#### Scheme 2

In scheme 1, the final or penultimate step was an oxidative cyclisation. An alternative, using thioacetal coupling, is shown in scheme 2. Mercury-mediated unmasking causes cyclisation to the protected PBD compound (A).

The thioacetal compound may be prepared as shown in scheme 2: the thioacetal protected C-ring [prepared via a literature method: Langley, D.R. & Thurston, D.E., J. Organic Chemistry, 52, 91-97 (1987)] is coupled to the o-nitrobenzoic acid (or alkyl ester after hydrolysis) (G) using a literature procedure. The

resulting nitro compound cannot be reduced by hydrogenation, because of the thioacetal group, so the tin(II) chloride method is used to afford the amine. This is then N-protected, e.g., by reaction with a chloroformate or acid chloride, such as 2,2,2-trichloroethylchloroformate.

Acetal-containing C-rings can be used as an alternative in this type of route with deprotection involving other methods, including the use of acidic conditions.

#### Dimer Synthesis (Scheme 3)

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$$\begin{array}{c} R_{0} \\ R_{0} \\$$

Scheme 3

PBD dimers may be synthesized using the strategy developed for

the synthesis of the protected PBD monomers. The sysnthesis routes illustrated in scheme 3 show compounds when the dimer linkage is of the formula  $-O-(CH_2)_n-O-$ . The step of dimer formation is normally carried out to form a bis(nitro acid) G'. This compound can then be treated as compound G in either scheme 1 or scheme 2 above.

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The bis(nitro acid) **G'** may be obtained by nitrating (e.g. using 70% nitric acid) the bis(carboxylic acid). This can be synthesised by alkylation of two equivalents of the relevant benzoic acid with the appropriate diiodoalkane under basic conditions. Many benzoic acids are commercially available and others can be synthesised by conventional methods.

Alternatively, the relevant benzoic acid esters can be joined together by a Mitsunolo etherification with an appropriate alkanediol, followed by nitration, and then hydrolysis (not illustrated).

An alternative synthesis of the bis(nitro acid) involves oxidation of the bis(nitro aldehyde), e.g. with potassium permanganate. This can be obtained in turn by direct nitration of the bis(aldehyde), e.g. with 70% HNO<sub>3</sub>. Finally, the bis(aldehyde) can be obtained via the Mitsunobu etherification of two equivalents of the benzoic aldehyde with the appropriate alkanediol.

## Preferred Synthetic Strategies for Compounds of formula Ia

The synthesis route of scheme 1 is generally applicable to

compounds of formula Ia.

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C2/C3-endo-unsaturated PBDs of formula Ia may be synthesised from their N10-carbamate protected precursors. Typically, palladium catalysed removal of an allyl carbamate may be used to generate the N10-C11 imine without affecting the key C2-unsaturation. For example, if the N10-C11 imine/carbinolamine is protected by an Alloc group, the C2/C3-endo-unsaturation is maintained during the Alloc cleavage reaction.

The reduction of the nitro-compound **E** as shown in scheme 1 with tin (II) chloride in refluxing methanol leaves the C2/C3-unsaturation unaffected. The hydrazine/Raney nickel method would not be suitable due to the double bond.

The compound of formula  $\mathbf{F}$  may be used in its TBDMS protected form, and therefore a deprotection step has to be included to produce the amino-alcohol compound  $\mathbf{E}$ .

The TBDMS ether, which is the product of the coupling of TBDMS protected compound with the appropriate o-nitrobenzoyl cloride, can be treated with AcOH:THF:H<sub>2</sub>O (3:1:1). TBAF was found to be unsuitable for this transformation due to the rapid degradation of reaction products.

A class of requisite C-ring providing compounds  ${\bf F}$  can be obtained

as shown in scheme 4.

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Commercially available trans-4-hydroxy-L-proline F8 can be N-alloc protected to give the allyl carbamate F7 which can then be esterified using standard conditions. Hydride reduction of the ester F6 furnishes the diol F5. Selective TBDMS protection of the diol gives a silyl ether F4, which can then be oxidised, using either Swern or TPAP oxidation, to provide the ketone F3.

The ketone **F3** can then undergo a Wittig reaction to yield a mixture of the E/Z exo-esters **F2** which can then be converted to the C2/C3-endo compound **F1(Ia)** upon treatment with excess sodium hydride.

Palladium-mediated cleavage of the N-alloc protecting group (Dangles O.; Guibé, F.; Balavoine, G.; Lavielle, S.; Marquet, A.;

J. Org. Chem. **1987**, 52, 4984) yields the compound **F(Ia)**.

#### Alternative route to compounds of formula Ia

A more linear synthetic route to compound **B** of scheme 1 has been developed which enables larger scale production of the C2/C3-endo-unsaturated PBDs, and is shown in scheme 5.

Scheme 5

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The silyl protecting group may be cleaved in good yield by

treating B1(Ia) with AcOH:THF:H2O (3:1:1). The key C2/C3-endounsaturation present in B1(Ia) may be introduced directly by performing the Horner-Emmons reaction on a ketone of type B2.

Unlike the previous route (Scheme 4), the addition of extra NaH to ensure double-bond migration was not necessary for this substrate. Swern oxidation of the secondary alcohol B3 may be used to furnish the ketone B2. The carbamate protected aniline B3 may be prepared from the nitro compound B5 in two steps.

Firstly, the nitro group may be reduced to the aniline by employing the Raney nickel/hydrazine method because a compound of type B5 lacks C2-unsaturation. This method is more advantageous than the tin (II) chloride procedure since the product is easier to isolate. The aniline B4 may then be N-carbamate protected in high yield without significant carbonate formation at C2.

An amide of type **B5** may be synthesised by coupling an acid chloride of type **G** to the key amine **KEC5** (Scheme 6).

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Scheme 6

Overall, this route has several advantages over the previous route which results in the larger scale production of the C2/C3-endo-unsaturated PBDs. Firstly, catalytic hydrogenation of KEC4 allows large scale preparation of key intermediate KEC5.

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Secondly, the nitro reduction step may be carried out on an intermediate devoid of C2-unsaturation. Importantly, the double-bond migration observed during the Horner-Emmons reaction is spontaneous, so excess sodium hydride is not necessary. This double-bond migration has also been observed by other workers (Leimgruber, W.; Batcho, A. D.; Czajkowski, R. C. J. Am. Chem. Soc. 1968, 90, 5641).

parr-hydrogenation of KEC4, in order to cleave the Cbz protecting group, allowed the large scale synthesis of the key amino intermediate KEC5. The TBDMS ether KEC4 was prepared in an analogous fashion to the corresponding Alloc protected intermediate F4 (Scheme 4). Selective silylation of the primary alcohol KEC3 was achieved using DBU as a silyl transfer agent. The diol KEC3 was obtained from hydride reduction of ester KEC2 which in turn was synthesised from carboxylic acid KEC1. N-Cbz protection of trans-4-hydroxy-L-proline (F4) was achieved by adopting a procedure reported in the literature (Bridges, R. J.; Stanley, M. S.; Anderson, M. W.; Cotman, C. W.; Chamberlain, R. A. J. Med. Chem. 1991, 34, 717).

Certain  $R_2$  groups may require protection during the synthesis 25 routes set out above, e.g. alcohols can be protected by using an acetate protecting group (see example 1(d)) The alternative synthesis routes are equally applicable to the synthesis of dimers.

<u>Preferred Synthesis Strategies for Compounds of formula II</u>

The synthesis route of scheme 1 is generally applicable to

5 compounds of formula II.

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C2-unsaturated PBDs of formula II may be synthesised from their N10-carbamate protected precursors. Typically, palladium catalysed removal of an allyl carbamate may be used to generate the N10-C11 imine without affecting the key C2-unsaturation. Alternatively, cadmium-lead couple may be employed to cleave an N10-2,2,2-trichloroethyl carbamate from the protected PBD.

The reduction of the nitro-compound  ${\bf E}$  as shown in scheme 1 with tin (II) chloride maintains the C2-unsaturation, although isolating the aniline  ${\bf C}$  from the tin salts can be problematic.

The compound of formula **F** may be used in its TBDMS protected form, and therefore a deprotection step has to be included to produce the amino-alcohol compound **E**.

The TBDMS ether of type E, which is the product of the coupling of the TBDMS protected compound with the appropriate onitrobenzoyl chloride, can be treated with AcOH: THF:  $\rm H_2O$  (3:1:1). TBAF was found to be unsuitable for this transformation due to the rapid degradation of reaction products.

C-ring providing compounds F(II) can be obtained as shown in scheme 7.

Scheme 7

- Commercially available trans-4-hydroxy-L-proline F8 can be N
  alloc protected to give the allyl carbamate F7 which can then be
  esterified using standard conditions. Hydride reduction of the
  ester F6 furnishes the diol F5. Selective TBDMS protection of
  the diol gives a silyl ether F4, which can then be oxidised,
  using either Swern or TPAP oxidation, to provide the ketone F3.
- 10 The C2-olefinic functionality present in F2 may be introduced by performing the Wittig reaction on ketone F3. Palladium-mediated

cleavage of the N-alloc protecting group (Dangles O.; Guibé, F.; Balavoine, G.; Lavielle, S.; Marquet, A.; J. Org. Chem. 1987, 52, 4984) yields compound F(II).

#### Alternative route to compound C

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Scheme 8

An alternative route to compound C has been developed (Scheme 8). The amide of formula C1 may be synthesised by forming the acid chloride of an N-Troc protected anthranilic acid of type C2. Interestingly, N-Troc anthranilic acids do not generate isatoic anhydrides, thus enabling amide formation reactions with amines of type F(II). Simultaneous TBAF-mediated cleavage of the 2,2,2-trichloroethyl carbamate and TBDMS groups from C1 may provide the key amino-alcohol C.

### Alternative Route to compounds of formula II

Scheme 9

A more linear synthetic route to compound B of scheme 1 has been developed which enables larger scale production of the C2-unsaturated PBDs, and is shown in scheme 9. TBAF-mediated cleavage of the TBDMS group may be used to produce B(II) from

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B1(II). The key C2-unsaturation present in B1(II) may be introduced by performing the Wittig olefination reaction on a ketone of type B2. Swern oxidation of the secondary alcohol B3 may be used to furnish the ketone B2. The carbamate protected aniline B3 may be prepared from the nitro compound B5 in two steps. Firstly, the nitro group may be reduced to the aniline by employing the Raney nickel/hydrazine method because a compound of type B5 lacks C2-unsaturation. This method is more advantageous than the tin (II) chloride procedure since the product is easier to isolate. The aniline B4 may then be N-carbamate protected in high yield without significant carbonate formation at C2.

An amide of type **B5** may be synthesised by coupling an acid chloride of type **G** to the key amine **KEC5** (see scheme 6).

Overall, this route has several advantages over the convergent route which allow larger scale production of the C2-unsaturated PBDs. Firstly, catalytic hydrogenation of **KEC4** allows large scale preparation of key intermediate **KEC5**. Secondly, the nitro reduction step may be carried out on an intermediate devoid of C2-unsaturation. Finally, the Wittig olefination may be performed in the latter stages of the synthetic route where large scale limitations are tolerated.

In dimer synthesis, the routes set out above may be used in preference to those set out in the overall synthetic strategies. In particular, the nitrogen-protecting group may advantageously be a carbamate, as protecting groups of this type may be removed in the final step by a variety of methods which, in general, do

not affect the key C2-unsaturation.

#### General Experimental Methods

Melting points (mp) were determined on a Gallenkamp P1384 digital melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded using a Perkin-Elmer 297 spectrophotometer.  $^{1}\mathrm{H-}$  and  $^{13}\mathrm{C-}$  NMR spectra were recorded on a Jeol GSX 270 MHZ FT-NMR spectrometer operating at 20°C +/-1°C. Chemical shifts are reported in parts per million  $(\delta)$  downfield from tetramethylsilane (TMS). Spin multiplicities are described as: s (singlet), bs (broad singlet), d (doublet), dd (doublet of 10 doublets), t (triplet), q (quartet), p (pentuplet) or m (multiplet). Mass spectra (MS) were recorded using a Jeol JMS-DX 303 GC Mass Spectrometer (EI mode: 70eV, source 117-147°C). Accurate molecular masses (HRMS) were determined by peak matching using perfluorokerosene (PFK) as an internal mass marker, and FAB 15 mass spectra were obtained from a qlycerol/thioglycerol/trifluoroacetic acid (1:1:0.1) matrix with a source temperature of 180°C. Optical rotations at the Na-D line were obtained at ambient temperature using a Perkin-Elmer 141 Polarimeter. Analytical results were generally within +/-20 0.2% of the theoretical values. Flash chromatography was performed using Aldrich flash chromatography "Silica Gel-60" (E. Merck, 230-400 mesh). Thin-layer chromatography (TLC) was performed using  $\operatorname{GF}_{254}$  silica gel (with fluorescent indicator) on glass plates. All solvents and reagents, unless otherwise 25 stated, were supplied by the Aldrich Chemical Company Ltd. and were used as supplied without further purification. Anhydrous

solvents were prepared by distillation under a dry nitrogen atmosphere in the presence of an appropriate drying agent, and were stored over  $4\text{\AA}$  molecular sieves or sodium wire. Petroleum ether refers to the fraction boiling at  $40\text{--}60^{\circ}\text{C}$ .

#### 5 Examples

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## Example 1(a): Synthesis of the 2-Cyanomethyl PBD (10, SB-A67) (see Figure 1)

#### Synthesis of the Nitro Alcohol (3)

A solution of the acid 1 (3.03 g, 10 mmol, 1 equiv) in freshly distilled CH<sub>2</sub>Cl<sub>2</sub> (50 mL) was treated with oxalyl chloride (1.05 mL, 12 mmol, 1.2 equiv) under a nitrogen atmosphere and stirred. DMF (0.1 mL) was added and the solution effervesced. The reaction was allowed to stir overnight at RT. The following day the acid chloride solution was added dropwise over 2 h to a stirred mixture of the amine 2 (2.31 g, 10 mmol, 1 equiv) and TEA (3.48 mL, 25 mmol, 2.5 equiv) in freshly distilled CH<sub>2</sub>Cl<sub>2</sub> (30 mL) while the temperature was kept under 0°C, under a nitrogen atmosphere. The reaction mixture was then allowed to warm to RT and stirred overnight. The solution was washed with NaHCO<sub>3</sub> (100 mL), saturated NH<sub>4</sub>Cl (100 mL), H<sub>2</sub>O (100 mL), brine (100 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo to give a brown oil which was purified by flash chromatography (SiO<sub>2</sub>, EtOAc) and provided the coupled compound 3 (3.24 g, 6.28 mmol, 62.8%) as a

vellow glass:  $^{1}$ H NMR (CDCl<sub>3</sub>, 270 MHz) rotamers:  $\delta$  -0.10 (s, 6H,  $Si(CH_3)_2$ , 0.80 (s, 9H,  $SiC(CH_3)_3$ , 2.04-2.55 (m, 3H, 1-H, OH), 3.05-4.60 (m, 9H, 11-H, 11a-H, OMe, 3-H, 2-H), 5.20 (br s, 2H, OBn), 6.78 and 6.85 (2xs, 1H, 6-H), 7.27-7.47 (m, 5H, Ph), 7.73 and 7.76 (2xs, 1H, 9-H);  $^{13}$ C NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  -5.5, -5.4, 5 18.2, 25.7, 25.8, 36.3, 56.6, 57.2, 62.6, 70.2, 71.3, 109.0, 109.4, 127.6-128.8, 135.2, 137.3, 147.9, 154.7, 166.6; IR (neat): 3401, 3065, 3033, 2951, 2856, 2739, 2628, 1956, 1743, 1620, 1578, 1522, 1462, 1434, 1378, 1336, 1277, 1221, 1075, 1051, 1002, 914, 836, 779, 752, 697, 669, 650, 615; EIMS m/z (relative intensity) 10 516 (M<sup>+</sup>, 0.6), 460 (29.8), 459 (92.6), 368 (7.9), 286 (49.6), 91 (100.0), 73 (9.5); FAB m/z (relative intensity) 517 (M<sup>+</sup>· +1, 15.1), 385 (9.2), 286 (19.3), 92 (9.3), 91 (100.0), 75 (14.0), 73 (42.2).

#### 15 Reduction to the Amino Alcohol (4)

A solution of hydrazine (3.11 mL, 100 mmol, 5 equiv) in MeOH (50 mL) was added dropwise to a refluxing solution of the nitro compound 3 (10.32 g, 20 mmol, 1 equiv), antibumping granules and Raney Ni (3.5 g) in MeOH (150 mL). After 1 h at reflux TLC (SiO<sub>2</sub>, 5% MeOH-CHCl<sub>3</sub>) revealed total consumption of starting material. The mixture was then treated with sufficient Raney Ni to decompose any unreacted hydrazine. After cooling to RT the mixture was filtered through Celite and the filtrate evaporated in vacuo. The resulting residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (300 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo to provide 4 (6.80 g, 14 mmol, 70%) as a pink oil which was carried through to the next stage without purification: <sup>1</sup>H NMR (CDCl<sub>3</sub>,

270 MHz) rotamers: δ -0.001 (s, 6H, Si(CH<sub>3</sub>)<sub>2</sub>), 0.88 (br s, 9H, SiC(CH<sub>3</sub>)<sub>3</sub>), 1.96-2.23 (m, 2H, 1-H), 3.44-4.48 (m, 12H, 11-H, 3-H, OMe, NH<sub>2</sub>, OH, 2-H, 11a-H), 5.09 (br s, 2H, OBn), 6.25 and 6.27 (2xs, 1H, 6-H), 6.68 and 6.73 (2xs, 1H, 9-H), 7.26-7.42 (m, 5H, Ph); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 270 MHz): δ -5.4, 18.2, 25.9, 35.7, 56.9, 57.2, 70.4, 70.7, 103.2, 112.9, 113.4, 127.2, 127.4, 127.9, 128.6, 128.6, 136.7, 141.6; IR (neat): 3356.80, 2930.13, 2857.36, 2247.82, 1622.19, 1514.60, 1463.60, 1408.95, 1261.43, 1176.55, 1118.48, 1003.88, 911.00, 836.61, 778.15, 733.59, 697.72, 646.32.

### 10 Synthesis of the Alloc Pro-N10-Protected C2-Alcohol (5)

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A solution of allyl chloroformate (1.54 mL, 14.48 mmol, 1.05 equiv) in freshly distilled  $\mathrm{CH_2Cl_2}$  (30 mL) was added dropwise to a stirred mixture of the amine 4 (6.70 g, 13.79 mmol, 1 equiv), pyridine (2.45 mL, 30.34 mmol, 2.2 equiv) in freshly distilled CH<sub>2</sub>Cl<sub>2</sub> (200 mL), at 0°C under a nitrogen atmosphere. The mixture 15 was allowed to warm at RT and stirred overnight. The following day TLC (SiO<sub>2</sub>, 5% MeOH-CHCl<sub>3</sub>) revealed reaction completion. mixture was washed with saturated  $CuSO_4$  (100 mL),  $H_2O$  (100 mL), brine (100 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo 20 to give a dark yellow oil. Flash chromatography (SiO2, 30% EtOAc-petroleum ether) afforded the pure Alloc-compound 5 (6.70 g, 11.75 mmol, 85.2%) as a yellow oil:  ${}^{1}H$  NMR (CDCl<sub>3</sub>, 270 MHz) rotamers:  $\delta$  0.03 and 0.04 (2xs, 6H, Si(CH<sub>3</sub>)<sub>2</sub>), 0.89 (br s, 9H,  $SiC(CH_3)_3$ , 1.99-2.40 (m, 2H, 1-H), 3.56 (br s, 4H, 11-H, 3-H), 25 3.79 (s, 3H, OMe), 4.05-4.20 (m, 1H, 11a-H), 4.38 (s, 1H, 2-H), 4.58-4.62 (m, 3H, OH, Alloc), 5.16-5.37 (m, 4H, OBn, Alloc), 5.86-6.00 (m, 1H, Alloc), 6.80 (s, 1H, 6-H), 7.30-7.48 (m, 5H,

#### Oxidation to the C2-Ketone (6)

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A solution of DMSO (2.50 mL, 35.25 mmol, 3 equiv) in freshly distilled CH2Cl2 (200 mL) was added dropwise over 1.5 h to a stirred solution of oxalyl chloride (8.81 mL of a 2M solution in CH<sub>2</sub>Cl<sub>2</sub>, 17.62 mmol, 1.5 equiv) at -55/-60°C (liquid nitrogen/CHCl<sub>3</sub>) under a nitrogen atmosphere. After 30 min stirring at -55°C, a solution of the secondary alcohol 5 (6.70 g, 11.75 mmol, 1 equiv) in  $CH_2Cl_2$  (150 mL) was added dropwise to the reaction mixture over 1.5 h. Following stirring at -55/-60 °C for 45 min the reaction was treated dropwise with a solution of TEA (11.14 mL, 79.90 mmol, 6.8 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) over a period of 40 min. The mixture was stirred for a further 45 min at -30°C and was then allowed to warm to RT. The reaction was then treated with brine (150 mL), cooled to 0°C and acidified to pH=2 with concentrated HCl. The organic phase was washed with  $H_2O$ (150 mL), brine (150 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo to give the ketone 6 as a dark orange oil (6.18 g, 10.88

mmol, 93%), sufficiently pure by TLC (SiO2, 40% EtOAc-petroleum ether) to be carried through to the next stage without further purification: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz) rotamers: δ 0.04 and 0.05 (2xs, 6H, Si(CH<sub>3</sub>)<sub>2</sub>), 0.87 (s, 9H, SiC(CH<sub>3</sub>)<sub>3</sub>), 2.47-2.78 (m, 2H,1-H), 3.66-4.10 (m, 8H, 3-H, OMe, 11-H, 11a-H), 4.62-4.65 (m, 2H, 5 Alloc), 4.80-5.40 (m, 4H, OBn, Alloc), 5.88-6.03 (m, 1H, Alloc), 6.76 (s, 1H, 6-H), 7.27-7.49 (m, 5H, Ph), 7.90 (s, 1H, 9-H), 8.62 (br s, 1H, NH);  $^{13}$ C NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  -5.8, -5.7, 18.0, 25.6, 25.7, 56.5, 65.8, 68.0, 70.7, 106.4, 111.0, 118.2, 127.7-128.6, 132.4, 136.1, 150.6, 153.4, 208.9; IR (neat): 3510, 3332, 10 2957, 2870, 2740, 1959, 1771, 1738, 1633, 1537, 1428, 1274, 1233, 1120, 1029, 844, 785, 700; EIMS m/z (relative intensity) 568  $(M^{+}, 90.6), 512 (28.7), 511 (79.8), 453 (12.1), 340 (38.6), 298$ (12.7), 282 (16.9), 172 (23.9), 91 (100.0), 41 (15.1);  $[\alpha]^{23}_{D} = 1.98^{\circ}$  (c = 1.010, CHCl<sub>3</sub>). 15

#### Insertion of the C2-Cyanomethyl Group (7)

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Sodium hydride (0.70 g of a 60% dispersion in mineral oil, 17.60 mmol, 2.5 equiv) was stirred in petroleum ether for 10 min. The suspension was allowed to settle and the solvent transferred under nitrogen from the flask via a double-tipped needle. The remaining residue was suspended in freshly distilled anhydrous THF (50 mL), cooled to 0°C and treated dropwise with a solution of the diethyl cyanomethylphosphonate (11.14 mL, 79.90 mmol, 6.8 equiv) in THF (60 mL) under a nitrogen atmosphere. The mixture was allowed to warm to RT and stir for 1.5 h. After cooling to 0°C the reaction mixture was treated dropwise with a solution of the ketone 6 (11.14 mL, 79.90 mmol, 6.8 equiv) in THF (40 mL).

After stirring overnight TLC (SiO2, 30% EtOAc-petroleum ether) revealed almost complete consumption of starting material. was evaporated in vacuo and the resulting residue treated with a saturated solution of NaHCO3 (100 mL) and EtOAc (100 mL). aqueous layer was washed with EtOAc (100 mL) and the combined 5 organic layers were then washed with H2O (100 mL), brine (100 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo to give a brown glass which was subjected to flash chromatography (SiO2, 30% EtOAc-petroleum ether) to provide the pure cyano compound 7 (2.6 g, 4.40 mmol, 63%) as a yellow glass: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 10 MHz):  $\delta$  0.03-0.09 (m, 6H, Si(CH<sub>3</sub>)<sub>2</sub>), 0.88 (m, 9H, SiC(CH<sub>3</sub>)<sub>3</sub>), 2.68-2.91 (m, 2H, 1-H), 3.12-3.13 (m, 2H, 12-H), 3.72-3.76 (m, 2H, 11-H), 3.82 (s, 3H, OMe), 4.62-4.65 (m, 2H, Alloc), 4.75 (m, 1H, 11a-H), 5.19 (s, 2H, OBn), 5.22-5.39 (m, 2H, Alloc), 5.88-6.02 (m, 1H, Alloc), 6.59 (s, 1H, 3-H), 6.68 (s, 1H, 6-H), 7.32-15 7.50 (m, 5H, Ph), 7.95 (s, 1H, 9-H), 8.72 (s, 1H, NH);  $^{13}$ C NMR  $(CDCl_3, 270 \text{ MHz}): \delta -5.4, 17.5, 18.1, 25.6-25.7, 34.0, 56.6,$ 59.8, 62.3, 65.8, 70.7, 106.1, 111.8, 114.0, 116.2, 118.1, 127.7-129.3, 132.4, 132.8, 136.1, 144.2, 150.9, 153.4, 166.1; IR (neat): 3337, 3067, 3034, 2954, 2930, 2857, 2253, 1732, 1622, 20 1599, 1524, 1495, 1464, 1408, 1362, 1336, 1259, 1205, 1166, 1116, 1051, 1026, 992, 914, 839, 778, 735, 698, 647; EIMS m/z (relative intensity) 591 (M<sup>+</sup>·, 20.1), 534 (15.0), 340 (67.5), 282 (20.9), 252 (25.6), 195 (32.4), 91 (100.0); HRMS m/z Calcd for 591.2765  $(C_{32}H_{41}N_3O_6Si)$ . Found 591.2758;  $[\alpha]^{23}D = -83.25^{\circ}$  (c = 1.015, 25 CHCl3).

#### Deprotected Alcohol (8)

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Glacial AcOH (15 mL) was added to a stirred solution of the silyl ether **7** (2.10 g, 3.55 mmol) in THF (10 mL) and  $H_2O$  (15 mL). reaction mixture was allowed to stir at RT and monitored every hour by TLC ( $SiO_2$ , 30% EtOAc-petroleum ether). Over the course 5 of 3 h AcOH (10 mL) was added in two further portions. mixture was stirred for a total of 4 h at which time the reaction had gone to completion. The mixture was then cooled to 0°C and treated dropwise with a 10% solution of  $NaHCO_3$  in  $H_2O$  (50 mL). The aqueous solution was extracted with EtOAc (3x20 mL) and the 10 combined organic layers were washed with H2O (20 mL), brine (20 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo to give a yellow oil. Flash chromatography (SiO2, 5% MeOH-CHCl3) afforded the free alcohol 8 (1.40 g, 2.93 mmol, 83%) as a yellow glass:  ${}^{1}H$  NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  2.41-3.02 (m, 2H, 1-H), 3.13 (s, 2H, 12-H), 3.70-4.10 (m, 6H, 11-H, OMe, OH), 4.61-4.64 (m, 2H, Alloc), 4.76 (m, 1H, 11a-H), 5.16 (s, 2H, OBn), 5.23-5.28 (m, 2H, Alloc), 5.87-6.02 (m, 1H, Alloc), 6.53 (s, 1H, 3-H), 6.78 (s, 1H, 6-H), 7.27-7.48 (m, 5H, Ph), 7.75 (s, 1H, 9-H), 8.45 (s, 1H, NH);  $^{13}$ C NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  17.4, 34.8, 56.8, 61.5, 65.1, 65.9, 70.8, 106.9, 111.8, 114.4, 116.1, 118.2, 127.7-129.1, 132.1, 132.4, 136.0, 144.8, 151.1, 153.7, 167.3; IR (neat): 3340, 3067, 2934, 2856, 2252, 1732, 1601, 1523, 1455, 1407, 1374, 1336, 1226, 1167, 1111, 1048, 1028, 996, 938, 869, 838, 768, 745, 698, 668, 636, 608; EIMS m/z (relative intensity) 477 (M<sup>+</sup>, 14.6), 340 (46.9), 282 (13.0), 91 (100.0); HRMS m/z Calcd for 477.1900  $(C_{26}H_{27}N_3O_6$ . Found 477.1962;  $[\alpha]^{23}_D = -67.42^{\circ}$  (c = 1.068, CHCl<sub>3</sub>).

#### N10-Protected Cyclized PBD (9)

A solution of DMSO (0.75 mL, 10.55 mmol, 3.6 equiv) in freshly distilled  $CH_2Cl_2$  (40 mL) was added dropwise at a rapid rate to a stirred solution of oxalyl chloride (2.64 mL of a 2M solution in  $CH_2Cl_2$ , 5.27 mmol, 1.8 equiv) at -40/-50°C (liquid 5 nitrogen/chlorobenzene) under a nitrogen atmosphere. After 5 min stirring at  $-45^{\circ}$ C, a solution of the primary alcohol 8 (1.40 g, 2.93 mmol, 1 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) was added dropwise to the reaction mixture over 45 min. Following stirring at -45°C for 45 10 min the reaction was treated dropwise with a solution of TEA (1.72 mL, 12.31 mmol, 4.2 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) over a period The mixture was stirred for a further 40 min at -45°C and was then allowed to warm to RT and diluted with 20 mL CH<sub>2</sub>Cl<sub>2</sub>. The reaction was then cooled to 0°C and washed with 1N HCl (200 mL),  $H_2O$  (100 mL), brine (100 mL), dried (MgSO<sub>4</sub>), filtered 15 and evaporated in vacuo to give a yellow foam which was subjected to flash chromatography (SiO2, 5% MeOH-CHCl3) to provide the pure ring closed compound 9 (0.95 g, 2.00 mmol, 68%) as a slightly yellow glass:  ${}^{1}H$  NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  2.69-3.14 (m, 2H, 1-H), 3.24 (s, 2H, 12-H), 3.84-3.98 (m, 6H, 11-H, OMe, OH), 4.46 (m, 20 2H, Alloc), 5.07-5.18 (m, 4H, OBn, Alloc), 5.60-5.80 (m, 2H, Alloc, 11a-H), 6.74 (s, 1H, 3-H), 7.04 (s, 1H, 6-H), 7.24-7.43 (m, 6H, Ph, 9-H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 270MHz):  $\delta$  17.5, 36.5, 56.2, 59.6, 66.9, 71.1, 85.7, 111.0, 113.2, 114.7, 116.1, 118.3, 124.6, 127.3-128.7, 131.7, 136.0, 149.2, 150.6, 163.6; IR (neat): 3396, 25 3089, 2938, 2615, 2251, 1707, 1602, 1513, 1432, 1308, 1219, 1113, 1045, 918, 869, 790, 735, 698, 648; EIMS m/z (relative intensity) 475 (M<sup>+</sup>·, 34.2), 340 (25.4), 339 (35.0), 279 (10.3), 134 (10.6),

91 (100.0); HRMS m/z Calcd for 475.1743 ( $C_{26}H_{25}N_3O_6$ ). Found 475.1883;  $[\alpha]^{23}_D = +101.46^{\circ}$  (c = 1.030, CHCl<sub>3</sub>).

#### C2-Cyanomethyl PBD (10, SB-A67)

Triphenylphosphine (25 mg, 0.095 mmol, 0.05 equiv), pyrrolidine (167  $\mu$ l, 2.0 mmol, 1.05 equiv) and Pd(PPh<sub>3</sub>)<sub>4</sub> (56 mg, 0.048 mmol, 5 0.025 equiv) were added sequentially to a stirred solution of the Alloc-compound 9 (900 mg, 1.90 mmol, 1 equiv) in freshly distilled dry CH<sub>2</sub>Cl<sub>2</sub> (100 mL). The reaction mixture was allowed to stir at RT under a nitrogen atmosphere for 2 h by which time TLC (SiO<sub>2</sub>, 1% MeOH-CHCl<sub>3</sub>) revealed reaction completion. 10 mixture was evaporated in vacuo and the residue applied to a gravity chromatography column (SiO2, 1% MeOH-CHCl3) to isolate the PBD SB-A67 (720 mg, 1.93 mmol, 100%) as a yellow glass:  $^{1}H$ NMR (CDCl<sub>3</sub>, 270 MHz): 3.05-3.40 (m, 4H, 1-H, 12-H), 3.95 (s, 3H, OMe), 4.38 (m, 1H, 11a-H), 5.21 (s, 2H, OBn), 6.84 (s, 1H, 6-H), 15 7.06 (s, 1H, 3-H), 7.27-7.70 (m, 6H, Ph, 9-H), 7.80 (d, 1H, 11a-H, J = 3 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  17.4, 36.8, 53.9, 56.3, 70.9, 111.7, 111.9, 112.8, 116.0, 118.7, 120.7, 127.1-128.7, 132.0, 136.0, 140.2, 148.3, 151.2, 161.8; IR (neat): 3353, 2931, 2251, 2222, 1604, 1508, 1437, 1247, 1120, 1000, 913, 874, 724, 20 697, 542; EIMS m/z (relative intensity) 373 (M<sup>+</sup>, 9.8), 371 (24.4), 280 (12.5), 91 (100.0); HRMS m/z Calcd for 373.1426  $(C_{22}H_{19}N_{3}O_{3})$ . Found 373.1364;  $[\alpha]^{23}_{D} = 254.5^{\circ}$  (c = 1.045, CHCl<sub>3</sub>).

Example 1(b): Synthesis of the 2-Methoxycarbonylmethyl PBD (5, SJG-245) (see Figure 2)

(2S,4R)-N-(Allyloxycarbonyl)-4-hydroxypyrrolidine-2-carboxylic acid (12)

A solution of allyl chloroformate (29.2 mL, 33.2 g, 275 mmol) in 5 THF (30 mL) was added dropwise to a suspension of trans-4hydroxy-L-proline (11) (30 g, 229 mmol) in a mixture of THF (150 mL) and  $H_2O$  (150 mL) at  $0^{\circ}C$  (ice/acetone), whilst maintaining the pH at 9 with 4 N NaOH. After stirring at 0°C for 1 h at pH 9, the aqueous layer was saturated with NaCl, and the 10 mixture diluted with EtOAc (100 mL). The aqueous layer was separated, washed with EtOAc (100 mL) and the pH adjusted to 2 with conc. HCl. The resulting milky emulsion was extracted with EtOAc (2 X 100 mL), washed with brine (200 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo to give the allyl carbamate 12 15 as a clear viscous oil (42.6 g, 87%):  $[\alpha]_{D}^{20} = -62.1$  ° (c = 0.69, MeOH);  ${}^{1}$ H NMR (270 MHz, CDCl<sub>3</sub> + DMSO- $d_{6}$ ) (Rotamers)  $\delta$  5.98-5.81  $(m, 1H, NCO_2CH_2CH=CH_2), 5.40-5.14 (m, 2H, NCO_2CH_2CH=CH_2), 4.64-$ 4.42 (m, 4H,  $NCO_2CH_2CH=CH_2$ ,  $NCH_2CHOHCH_2$  and  $CHCO_2H$ ), 3.82-3.51 (m, 2H,  $NCH_2CHOHCH_2$ ), 2.34-2.08 (m, 2H,  $NCH_2CHOHCH_2$ ); <sup>13</sup>C NMR (67.8) 20 MHz, CDCl $_3$  + DMSO) (Rotamers)  $\delta$  175.0 and 174.5 ( $CO_2H$ ), 155.1 and 154.6 (NC=0), 132.9 and 132.8 (NCO<sub>2</sub>CH<sub>2</sub>CH=CH<sub>2</sub>), 117.6 and 116.7  $(NCO_2CH_2CH=CH_2)$ , 69.5 and 68.8  $(NCH_2CHOH)$ , 65.9 and 65.8

 $(NCO_2CH_2CH=CH_2)$ , 58.0 and 57.7  $(CHCO_2H)$ , 55.0 and 54.5  $(NCH_2CHOH)$ , 39.3 and 38.3  $(NCH_2CHOHCH_2)$ ; MS (EI), m/z (relative intensity) 215  $(M^+$ , 10) 197(12), 170  $(M-CO_2H, 100)$ , 152 (24), 130  $(M-CO_2C_3H_5, 97)$ , 126 (34), 112 (50), 108 (58), 86 (11), 68 (86), 56 (19); IR (Neat) 3500-2100 (br, OH), 2950, 1745 and 1687 (br, C=O), 1435, 1415, 1346, 1262, 1207, 1174, 1133, 1082, 993, 771  $cm^{-1}$ ; exact mass calcd for  $C_9H_{13}NO_5$  m/e 215.0794, obsd m/e 215.0791.

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## Methyl (2S, 4R) -N-(Allyloxycarbonyl) -4-hydroxypyrrolidine-2-carboxylate (13)

A catalytic amount of concentrated  $H_2SO_4$  (4.5 mL) was added to a solution of Alloc-hydroxyproline (12) (43 g, 200 mmol) in MeOH (300 mL) at 10°C (ice) and the reaction mixture was then heated at reflux for 2 h. After cooling to room temperature the reaction mixture was treated with TEA (43 mL) and the MeOH 15 evaporated in vacuo. The residue was dissolved in EtOAc (300 mL), washed with brine (200 mL), dried (MgSO<sub>4</sub>), filtered and concentrated in vacuo to give a viscous oil. Purification by flash chromatography (40% EtOAc/Petroleum Ether) removed the high R<sub>f</sub> impurity to provide the pure ester 13 as a transparent yellow 20 oil (19.6 g, 43%):  $[\alpha]^{23}_{D} = -79.0$ ° (c = 0.35, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) (Rotamers)  $\delta$  5.98-5.78 (m, 1H, NCO<sub>2</sub>CH<sub>2</sub>CH=CH<sub>2</sub>), 5.35-5.16 (m, 2H,  $NCO_2CH_2CH=CH_2$ ), 4.65-4.45 (m, 4H,  $NCO_2CH_2CH=CH_2$ ,  $NCH_2CHOHCH_2$  and  $NCHCO_2CH_3$ ), 3.75 and 3.72 (s x 2, 3H,  $OCH_3$ ), 3.70-3.54 (m, 2H,  $NCH_2CHOHCH_2$ ), 3.13 and 3.01 (br s x 2, 1H, OH), 25 2.39-2.03 (m, 2H,  $NCH_2CHOHCH_2$ ); <sup>13</sup>C NMR (67.8 MHz,  $CDCl_3$ ) (Rotamers)  $\delta$  173.4 and 173.2 ( $CO_2CH_3$ ), 155.0 and 154.6 (NC=O),

132.6 and 132.4 (NCO<sub>2</sub>CH<sub>2</sub>CH=CH<sub>2</sub>), 117.6 and 117.3 (NCO<sub>2</sub>CH<sub>2</sub>CH=CH<sub>2</sub>), 70.0 and 69.2 (NCH<sub>2</sub>CHOH), 66.2 (NCO<sub>2</sub>CH<sub>2</sub>CH=CH<sub>2</sub>), 57.9 and 57.7 (NCHCO<sub>2</sub>CH<sub>3</sub>), 55.2 and 54.6 (NCH<sub>2</sub>CHOH), 52.4 (OCH<sub>3</sub>), 39.1 and 38.4 (NCH<sub>2</sub>CHOHCH<sub>2</sub>); MS (EI), m/z (relative intensity) 229 (M<sup>+</sup>, 7), 170 (M-CO<sub>2</sub>Me, 100), 144 (M-CO<sub>2</sub>C<sub>3</sub>H<sub>5</sub>, 12), 126 (26), 108 (20), 68 (7), 56 (8); IR (Neat) 3438 (br, OH), 2954, 1750 and 1694 (br, C=O), 1435, 1413, 1345, 1278, 1206, 1130, 1086, 994, 771 cm<sup>-1</sup>; exact mass calcd for  $C_{10}H_{15}NO_5$  m/e 229.0950, obsd m/e 229.0940.

## (2S,4R)-N- (Allyloxycarbonyl) -4-hydroxy-2- (hydroxymethyl) pyrrolidine (14)

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A solution of the ester 13 (19.5 g, 85 mmol) in THF (326 mL) was cooled to 0°C (ice/acetone) and treated with LiBH<sub>4</sub> (2.78 g, 128 mmol) in portions. The reaction mixture was allowed to warm to room temperature and stirred under a nitrogen atmosphere for 2.5 h at which time TLC (50% EtOAc/Petroleum Ether) revealed complete consumption of ester 13. The mixture was cooled to 0°C and water (108 mL) was carefully added followed by 2 N HCl (54 mL). After evaporation of the THF in vacuo, the mixture was neutralised to pH 7 with 10 N NaOH and saturated with solid NaCl. The saturated aqueous solution was then extracted with EtOAc (5 X 100 mL), the combined organic layers washed with brine (200 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo to furnish the pure diol 14 as a clear colourless oil (16.97 g, 99%):  $[\alpha]_{D}^{24} = -57.0^{\circ}$  $(c = 0.61, CHCl_3);$  <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  6.01-5.87 (m, 1H,  $NCO_2CH_2CH=CH_2$ ), 5.36-5.20 (m, 2H,  $NCO_2CH_2CH=CH_2$ ), 4.84 (br s, 1H,  $NCHCH_2OH)$ , 4.60 (d, 2H, J = 5.31 Hz,  $NCO_2CH_2CH=CH_2$ ), 4.39 (br s, 1H, NCHCH2OH), 4.18-4.08 (m, 1H, 3.35, NCH2CHOH), 3.90-3.35 (m,

4H, NC $H_2$ CHOH, NCHCH $_2$ OH, and OH), 3.04 (br s, 1H, OH), 2.11-2.03 (m, 1H, NC $H_2$ CHOHC $H_2$ ), 1.78-1.69 (m, 1H, NC $H_2$ CHOHC $H_2$ ); <sup>13</sup>C NMR (67.8 MHz, CDCl $_3$ )  $\delta$  157.1 (NC=O), 132.6 (NCO $_2$ CH $_2$ CH=CH $_2$ ), 117.7 (NCO $_2$ CH $_2$ CH= $_2$ CH $_3$ ), 69.2 (NCH $_2$ CHOH), 66.4 and 66.2 (NCO $_2$ CH $_2$ CH=CH $_2$ ) and NCH $_2$ OH), 59.2 (N $_2$ CHOH), 55.5 (N $_2$ CHOH), 37.3 (NCH $_2$ CHOH $_3$ CHOH $_3$ CHOH $_4$ CHOH $_4$ CHO), 170 (M-CH $_3$ OH), 144 (M-OC $_3$ H $_5$ , 6), 126 (26), 108 (20), 68 (9); IR (Neat) 3394 (br, OH), 2946, 2870, 1679 (C=O), 1413, 1339, 1194, 1126, 1054, 980, 772 cm<sup>-1</sup>; exact mass calcd for C $_3$ H $_1$ 5NO $_4$  m/e 201.1001, obsd m/e 201.1028.

### (2S, 4R) -N- (Allyloxycarbonyl) -2- (tert-

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#### butyldimethylsilyloxymethyl)-4-hydroxypyrrolidine (15)

A solution of the diol 14 (16.97 g, 84 mmol) in  $CH_2Cl_2$  (235 mL) was treated with TEA (11.7 mL, 8.5 g, 84 mmol) and stirred for 15 min at room temperature. TBDMSCl (9.72 g, 64 mmol) and DBU (16.8 mmol, 2.51 mL, 2.56 g) were added and the reaction mixture stirred for a further 16 h under a nitrogen atmosphere. The reaction mixture was diluted with EtOAc (500 mL), washed with saturated NH<sub>4</sub>Cl (160 mL), brine (160 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo to give an oil which was a mixture of the required product (major component), unreacted diol and the presumed disilyated compound by TLC (50% EtOAc/Petroleum Ether). Flash chromatography (20-100% EtOAc/Petroleum Ether) isolated the 3 components, to provide the monosilylated compound 15 as a slightly yellow transparent oil (13.85 g, 52%):  $[\alpha]^{21}_{D} = -58.6^{\circ}$  (c = 1.14,  $CHCl_3$ ); <sup>1</sup>H NMR (270 MHz,  $CDCl_3$ ) (Rotamers)  $\delta$  6.01-5.86 (m, 1H,  $NCO_2CH_2CH=CH_2$ ), 5.34-5.18 (m, 2H,  $NCO_2CH_2CH=CH_2$ ), 4.59-

4.49 (m, 3H,  $NCO_2CH_2CH=CH_2$  and  $NCHCH_2OTBDMS$ ), 4.06-3.50 (m, 5H,  $NCH_2CHOH$ ,  $NCH_2CHOH$  and  $NCHCH_2OTBDMS$ ), 2.20-2.01 (m, 2H,  $NCH_2CHOHCH_2$ ), 0.87 (s, 9H,  $SiC(CH_3)_3$ ), 0.0 (s, 6H,  $Si(CH_3)_2$ ); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>) (Rotamers)  $\delta$  155.0 (NC=O), 133.1  $(NCO_2CH_2CH=CH_2)$ , 117.6 and 117.1  $(NCO_2CH_2CH=CH_2)$ , 70.3 and 69.7 5  $(NCH_2CHOH)$ , 65.9 and 65.6  $(NCO_2CH_2CH=CH_2)$ , 63.9 and 62.8 (NCH $CH_2$ OTBDMS), 57.8 and 57.4 (NCHCH $_2$ OTBDMS), 55.7 and 55.2  $(NCH_2CHOH)$ , 37.3 and 36.6  $(NCH_2CHOHCH_2)$ , 25.9  $(SiC(CH_3)_3)$ , 18.2  $(SiC(CH_3)_3)$ , -5.5  $(Si(CH_3)_2)$ ; MS (EI), m/z (relative intensity) 316  $(M^{+} + 1, 29)$ , 315  $(M^{+}, 4)$ , 300  $(M-CH_3, 26)$ , 284 (4), 261 10 (8), 260 (50), 259 (100), 258 (M-OC<sub>3</sub>H<sub>5</sub> or M- $^{t}$ Bu, 100), 218 (13), 215(10), 214 (52), 200 (12), 170 (M-CH<sub>2</sub>OTBDMS, 100), 156 (40), 126 (58), 115 (33), 108 (41), 75 (35); IR (Neat) 3422 (br, OH), 2954, 2858, 1682 (C=O), 1467, 1434, 1412 (SiCH<sub>3</sub>), 1358, 1330, 1255 (SiCH<sub>3</sub>), 1196, 1180, 1120, 1054, 995, 919, 837, 776, 669 cm<sup>-</sup> 15  $^{1}$ ; exact mass calcd for  $C_{15}H_{29}NO_{4}Si$  m/e 315.1866, obsd m/e 315.1946.

(2S) -N- (Allyloxycarbonyl) -2- (tert-butyldimethylsilyloxymethyl) -4- oxopyrrolidine (16).

Method A: A solution of DMSO (12.9 mL, 14.3 g, 183 mmol) in  $CH_2Cl_2$  (90 mL) was added dropwise to a solution of oxalyl chloride (45.1 mL of a 2.0 M solution in  $CH_2Cl_2$ , 90.2 mmol) at -60 °C (dry ice/acetone) under a nitrogen atmosphere. After stirring at -70 °C for 30 min, a solution of the alcohol 15 (25.8 g, 81.9 mmol) dissolved in  $CH_2Cl_2$  (215 mL) was added dropwise at -60°C. After 1.5 h at -70°C, the mixture was treated dropwise with TEA (57.2 mL, 41.5 g, 410 mmol) and allowed to warm

to 10°C. The reaction mixture was treated with brine (150 mL) and acidified to pH 3 with conc. HCl. The layers were separated and the organic phase washed with brine (200 mL), dried (MgSO $_4$ ), filtered and concentrated *in vacuo* to give an orange oil.

Purification by flash chromatography (40% EtOAc/Petroleum Ether) furnished the ketone **16** as a pale yellow oil (24.24 g, 95%):

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Method B: A solution of the alcohol 15 (4.5 g, 14.3 mmol) in  $CH_2Cl_2$  (67.5 mL) was treated with  $CH_3CN$  (7.5 mL), 4 Å powdered molecular sieves (3.54 g) and NMO (2.4 g, 20.5 mmol). After 15 min stirring at room temperature, TPAP (0.24 g, 0.7 mmol) was added to the reaction mixture and a colour change (green - black) was observed. The reaction mixture was allowed to stir for a further 2.5 h at which time complete consumption of starting material was observed by TLC (50% EtOAc/Petroleum ether 40 °-

15 60°). The black mixture was concentrated in vacuo and the pure ketone 16 was obtained by flash chromatography (50% EtOAc/Petroleum Ether) as a golden oil (4.1 g, 92%):  $\left[\alpha\right]^{22}_{D}$  = +1.25° (c = 10.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) (Rotamers)  $\delta$  6.0-5.90 (m, 1H, NCO<sub>2</sub>CH<sub>2</sub>CH=CH<sub>2</sub>), 5.35-5.22 (m, 2H, NCO<sub>2</sub>CH<sub>2</sub>CH=CH<sub>2</sub>),

20 4.65-4.63 (m, 2H,  $NCO_2CH_2CH=CH_2$ ), 4.48-4.40 (m, 1H,  $NCH_2OTBDMS$ ), 4.14-3.56 (m, 4H,  $NCH_2C=0$  and  $NCH_2OTBDMS$ ), 2.74-2.64 (m, 1H,  $NCH_2C=OCH_2$ ), 2.46 (d, 1H, J=18.69 Hz,  $NCH_2C=OCH_2$ ), 0.85 (s, 9H,  $SiC(CH_3)_3$ ), 0.0 (s, 6H,  $Si(CH_3)_2$ ); <sup>13</sup>C NMR (67.8 MHz,  $CDCl_3$ ) (Rotamers)  $\delta$  210.1 (C=0), 154.1 (NC=0), 132.7

 $(NCO_2CH_2CH=CH_2)$ , 118.0 and 117.7  $(NCO_2CH_2CH=CH_2)$ , 66.0 and 65.8  $(NCO_2CH_2CH=CH_2)$ , 65.0  $(NCHCH_2OTBDMS)$ , 55.7  $(NCHCH_2OTBDMS)$ , 53.6  $(NCH_2C=O)$ , 40.8 and 40.1  $(NCH_2C=OCH_2)$ , 25.7  $(SiC(CH_3)_3)$ , 18.1  $(SiC(CH_3)_3)$ , -5.7 and -5.8  $(Si(CH_3)_2)$ ; MS (CI), m/z (relative)

intensity) 314 (M<sup>+</sup> + 1, 100), 256 (M-OC<sub>3</sub>H<sub>5</sub> or M-<sup>t</sup>Bu, 65); IR (Neat) 2930, 2858, 1767 (C=O), 1709 (NC=O), 1409 (SiCH<sub>3</sub>), 1362, 1316, 1259 (SiCH<sub>3</sub>), 1198, 1169, 1103, 1016, 938, 873, 837, 778, 683 cm<sup>-1</sup>; exact mass calcd for  $C_{15}H_{27}NO_4Si$  m/e 313.1710, obsd m/e 313.1714.

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(2S) -N-(Allyloxycarbonyl) -2-(tert-butyldimethylsilyloxymethyl) -4-(methoxycarbonylmethyl) -2,3-dihydropyrrole (17).

Petroleum ether 40°-60° (100 mL) was added to a sample of NaH (0.80 g of a 60% dispersion in oil, 20.12 mmol) and stirred at room temperature under a nitrogen atmosphere. After 0.5 h the mixture was allowed to settle and the Petroleum Ether was transferred from the flask via a double-tipped needle under nitrogen. THF (100 mL) was added to the remaining residue and the mixture was cooled to 0°C (ice/acetone). The cool solution was treated dropwise with a solution of methyldiethylphosphonoacetate (3.69 mL, 4.23 g, 20.12 mmol) in THF (100 mL) under nitrogen. After 1 h at room temperature, the mixture was cooled to 0°C and treated dropwise with a solution of the ketone 16 (3.0 g, 9.58 mmol) in THF (30 mL) under nitrogen. After 16 h at room temperature, TLC (50% EtOAc/Petroleum Ether) revealed the complete consumption of ketone and further TLC (5% EtOAc/Petroleum Ether) revealed the formation of mainly the exoproduct. The reaction mixture was cooled to 0 °C (ice/acetone) and transferred via a double-tipped needle under nitrogen to another flask containing NaH (0.40 g of a 60% dispersion in oil, 10.1 mmol) at 0°C, freshly washed as above. The reaction mixture was maintained at 0 °C, and after 40 min TLC revealed the almost

complete conversion to endo-product. The THF was evaporated in vacuo and the mixture partitioned between saturated NaHCO3 (100 mL) and EtOAc (100 mL). The layers were separated and the aqueous layer extracted with EtOAc (2 X 50 mL). The combined organic layers were washed with brine (100 mL), dried (MgSO<sub>4</sub>), 5 filtered and concentrated in vacuo to give an orange oil. Purification by flash chromatography (5% EtOAc/Petroleum Ether) furnished the *endo-ester* **17** (2.22 g, 63%):  $[\alpha]^{21}_{n} = -97.7$  ° (c = 2.78, CHCl<sub>3</sub>);  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>) (Rotamers)  $\delta$  6.47 and 6.42 (br s x 2, 1H,  $NCH=CCH_2CO_2CH_3$ ), 5.98-5.86 (m, 1H,  $NCO_2CH_2CH=CH_2$ ), 10 5.31 (d, 1H, J = 16.85 Hz,  $NCO_2CH_2CH=CH_2$ ), 5.22 (d, 1H, J = 10.62Hz,  $NCO_2CH_2CH=CH_2$ ), 4.65-4.49 (m, 2H,  $NCO_2CH_2CH=CH_2$ ), 4.37-4.18 (m, 1H, NCHCH<sub>2</sub>OTBDMS), 3.76-3.69 (m, 5H, NCHCH<sub>2</sub>OTBDMS) and $CO_2CH_3$ ), 3.09 (br s, 2H, NCH=CC $H_2CO_2CH_3$ ), 2.86-2.80 (m, 1H,  $NCH=CCH_2CO_2CH_3CH_2$ ), 2.59 (d, 1H, J=17.40 Hz,  $NCH=CCH_2CO_2CH_3CH_2$ ), 15 0.87 (s, 9H, SiC(CH<sub>3</sub>)<sub>3</sub>), 0.04 and 0.03 (s x 2, 6H, Si(CH<sub>3</sub>)<sub>2</sub>);  $^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>) (Rotamers)  $\delta$  171.2 (CO<sub>2</sub>CH<sub>3</sub>), 151.9 (NC=O), 132.8 ( $NCO_2CH_2CH=CH_2$ ), 127.1 and 126.4 ( $NCH=CCH_2CO_2CH_3$ ), 118.0 and 117.7  $(NCO_2CH_2CH=CH_2)$ , 114.6  $(NCH=CCH_2CO_2CH_3)$ , 65.9  $(NCO_2CH_2CH=CH_2)$ , 63.4 and 62.6  $(NCH_2OTBDMS)$ , 59.0 and 58.7 20 (NCHCH<sub>2</sub>OTBDMS), 51.9 (CO<sub>2</sub>CH<sub>3</sub>), 36.0 and 34.8 (NCH=CCH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>CH<sub>2</sub>), 34.2 and 33.9 (NCH= $CCH_2CO_2CH_3$ ), 25.8 (SiC( $CH_3$ )<sub>3</sub>), 18.2  $(SiC(CH_3)_3)$ , -5.4 and -5.5  $(Si(CH_3)_2)$ ; MS (EI), m/z (relative intensity) 369 ( $M^{+}$ , 58), 354 (28), 326 (31), 312 ( $M-OC_3H_5$  or Mt<sub>Bu</sub>, 100), 268 (80), 236 (21), 227 (86), 210 (22), 192 (22), 168 25 (93), 152 (55), 138 (22), 120 (79), 89 (70), 73 (75); IR (NEAT) 3086, 2954, 2930, 2885, 2857, 1744, 1709, 1670, 1463, 1435, 1413,

1362, 1337, 1301, 1253, 1195, 1107, 1064, 1014, 983, 937, 887,

838, 778, 758, 680, 662 555 cm<sup>-1</sup>; exact mass calcd for  $C_{18}H_{31}NO_5Si$  m/e 369.1972, obsd m/e 369.1868.

# (2S) -2-(tert-butyldimethylsilyloxymethyl)-4(methoxycarbonylmethyl)-2,3-dihydropyrrole (18)

A catalytic amount of  $PdCl_2(PPh_3)_2$  (84 mg, 0.12 mmol) was added 5 to a stirred solution of the allyl carbamate 17 (1.10 g, 2.98 mmol) and  $H_2O$  (0.32 mL, 17.8 mmol) in  $CH_2Cl_2$  (36 mL). After 5 min stirring at room temperature,  $Bu_3SnH$  (0.89 mL, 0.96 g, 3.30 mmol) was added rapidly in one portion. A slightly exothermic reaction with vigorous gas evolution immediately ensued. The 10 mixture was stirred for 16 h at room temperature under nitrogen at which time TLC (50% EtOAc/Petroleum Ether) revealed the formation of amine along with the complete consumption of starting material. After diluting with  $CH_2Cl_2$  (30 mL), the organic solution was dried  $(MgSO_4)$ , filtered and evaporated in 15 vacuo to give an orange oil which was purified by flash chromatography (50% EtOAc/Petroleum Ether) to afford the enamine 18 as a slightly orange oil  $(0.57 \text{ g, } 67\%): ^{1}\text{H NMR}$  (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.53 and 7.48 (br s x 2, 1H, NCH=CCH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>), 4.35-4.13  $(m, 1H, NCHCH_2OTBDMS), 3.82-3.17 (m, 7H, NCH=CCH_2CO_2CH_3,$ 20  $NCHCH_2OTBDMS$  and  $CO_2CH_3$ ), 2.64-2.04 (m, 2H,  $NCH=CCH_2CO_2CH_3CH_2$ ), 0.90-0.88 (m, 9H, SiC(CH<sub>3</sub>)<sub>3</sub>), 0.09-0.00 (m, 6H, Si(CH<sub>3</sub>)<sub>2</sub>); MS (EI), m/z (relative intensity) 285 (M<sup>+</sup>·, 1), 270 (M-CH<sub>3</sub>, 7), 254 (6), 242 (4), 230 (6), 228 (M-<sup>t</sup>Bu, 100), 212 (4), 196 (3), 168 (13), 115 (3), 89 (10), 80 (4), 73 (13); MS (CI), m/z (relative 25 intensity) 342 ( $M^+$ : +57, 7), 302 ( $M^+$ : +17, 7), 286 ( $M^+$ : +1, 100), 228 (M-<sup>t</sup>Bu, 100).

(2S) -N- (4-Benzyloxy-5-methoxy-2-nitrobenzoyl) -2-(tert-butyldimethylsilyloxymethyl) -4-(methoxycarbonylmethyl) -2,3-dihydropyrrole (19).

A catalytic amount of DMF (2 drops) was added to a stirred solution of the acid 1 (0.506 g, 1.67 mmol) and oxalyl chloride 5 (0.17 mL, 0.25 g, 1.98 mmol) in  $CH_2Cl_2$  (33 mL). After 16 h at room temperature the acid chloride solution was added dropwise to a stirred mixture of the enamine 18 (0.524 g, 1.84 mmol) and TEA  $(0.47 \text{ g}, 0.65 \text{ mL}, 4.60 \text{ mmol}) \text{ in } CH_2Cl_2 \text{ (12 mL)} \text{ at } 0^{\circ}\text{C}$ (ice/acetone) under a nitrogen atmosphere. The reaction mixture 10 was allowed to warm to room temperature and stirred for a further The mixture was diluted with  $CH_2Cl_2$  (50 mL), washed with 2.5 h. saturated NaHCO $_3$  (50 mL), saturated NH $_4$ Cl (50 mL), H $_2$ O (50 mL), brine (50 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo to give the crude product as a dark orange oil. Purification by 15 flash chromatography (25% EtOAc/Petroleum Ether) isolated the pure enamide 19 as an orange oil (0.55 g, 58%): <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.77 (s, 1H<sub>arom</sub>), 7.45-7.28 (m, 5H<sub>arom</sub>), 6.81 (s, 1H<sub>arom</sub>), 5.80 (s, 1H, NCH=CCH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>), 5.22 (s, 2H, PhCH<sub>2</sub>O), 4.76-4.64 (m, 1H, NCHCH2OTBDMS), 3.97 (s, 3H, OCH3), 3.72-3.66 (m, 5H, 20  $NCHCH_2OTBDMS$  and  $CO_2CH_3$ ), 3.02 (s, 2H,  $NCH=CCH_2CO_2CH_3$ ), 3.01-2.63 (m, 2H, NCH=CCH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>CH<sub>2</sub>), 0.90 (s, 9H, SiC(CH<sub>3</sub>)<sub>3</sub>), 0.11 (s, 6H,  $Si(CH_3)_2$ ); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  170.7 ( $CO_2CH_3$ ), 154.6 (NC=O), 148.3 ( $C_{\text{arom}}$ ), 137.6 ( $C_{\text{arom}}$ ), 135.2 ( $C_{\text{arom}}$ ), 128.8, 128.5 and 127.6 (BnC-H<sub>arom</sub>), 126.7 (C<sub>arom</sub>), 126.1 (NCH=CCH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>), 118.8 25  $(NCH=CCH_2CO_2CH_3)$ , 109.9  $(C-H_{arom})$ , 109.0  $(C-H_{arom})$ , 71.3  $(PhCH_2O)$ , 60.7 (NCH $CH_2$ OTBDMS), 59.0 (NCHCH $_2$ OTBDMS), 56.7 (OCH $_3$ ), 52.0  $(CO_2CH_3)$ , 35.1  $(NCH=CCH_2CO_2CH_3)$ , 33.8  $(NCH=CCH_2CO_2CH_3CH_2)$ , 25.8

 $(SiC(CH_3)_3)$ , 18.2  $(SiC(CH_3)_3)$ , -5.3 and -5.4  $(Si(CH_3)_2)$ .

(2S) -N- (4-Benzyloxy-5-methoxy-2-nitrobenzoyl)-2- (hydroxymethyl)-4- (methoxycarbonylmethyl)-2,3-dihydropyrrole (20).

A solution of the silyl protected compound 274 (0.45 g, 0.79 mmol) in THF (8 mL) was treated with  $\rm H_2O$  (8 mL) and glacial 5 acetic acid (24 mL). After 5 h stirring at room temperature TLC (50% EtOAc/Petroleum Ether) showed the complete consumption of starting material. The mixture was carefully added dropwise to a stirred solution of  $NaHCO_3$  (64 g) in  $H_2O$  (640 mL) and extracted with EtOAc (3 X 100 mL). The combined organic layers were washed 10 with  $H_2O$  (100 mL), brine (100 mL), dried (MgSO<sub>4</sub>), filtered and concentrated in vacuo to give the crude product as an orange glass. Purification by flash chromatography (80% EtOAc/Petroleum Ether) furnished the pure alcohol 20 as a light orange glass (0.35 g, 98%): <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.78 (s, 1H<sub>arom</sub>), 7.48-15 7.33 (m,  $5H_{arom}$ ), 6.86 (s,  $1H_{arom}$ ), 5.82 (s, 1H,  $NCH=CCH_2CO_2CH_3$ ), 5.22 (s, 2H,  $PhCH_2O$ ), 4.81-4.71 (m, 1H,  $NCHCH_2OH$ ), 3.98-3.92 (m, 5H, NCHCH<sub>2</sub>OH and OCH<sub>3</sub>), 3.72 (s, 3H,  $CO_2CH_3$ ), 3.10-2.22 (m, 3H,  $NCH=CCH_2CO_2CH_3$  and  $NCH=CCH_2CO_2CH_3CH_2$ ), 2.50-2.35 (m, 1H,  $NCH=CCH_2CO_2CH_3CH_2$ ); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  170.6 ( $CO_2CH_3$ ), 20 154.8 (NC=O), 148.5 ( $C_{arom}$ ), 137.5 ( $C_{arom}$ ), 135.1 ( $C_{arom}$ ), 128.9, 128.6 and 127.6  $(BnC-H_{arom})$ , 126.2  $(NCH=CCH_2CO_2CH_3)$ , 119.4  $(NCH=CCH_2CO_2CH_3)$ , 109.8  $(C-H_{arom})$ , 109.0  $(C-H_{arom})$ , 71.4  $(PhCH_2O)$ , 61.5  $(NCHCH_2OH)$ , 61.4  $(NCHCH_2OH)$ , 56.8  $(OCH_3)$ , 52.1  $(CO_2CH_3)$ , 35.6  $(\text{NCH=CCH}_2\text{CO}_2\text{CH}_3)\,,\ 33.5\ (\text{NCH=CCH}_2\text{CO}_2\text{CH}_3\text{CH}_2)\,;\ \text{MS (EI)}\,,\ \textit{m/z}\ (\text{relative})$ 25 intensity) 456 ( $M^{+}$ , 7), 286 (M-NCHC= $CH_2CO_2CH_3CH_2CHCH_2OH$ , 25), 270  $(NCHC=CH_2CO_2CH_3CH_2CHCH_2OH, 6), 91 (PhCH_2, 100), 80 (6); exact mass$ 

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calcd for  $C_{23}H_{24}N_2O_8$  m/e 456.1533, obsd m/e 456.1557.

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(2S) -N- (2-Amino-4-benzyloxy-5-methoxybenzoyl) -2- <math>(hydroxymethyl) -4- (methoxycarbonylmethyl) -2, 3-dihydropyrrole (21).

A solution of the nitro-alcohol 20 (0.35 g, 0.77 mmol) and  $SnCl_2/2H_2O$  (0.87 g, 3.86 mmol) in methanol (16 mL) was heated to reflux and monitored by TLC (90% CHCl $_3$ /MeOH). After 1 h the MeOH was evaporated in vacuo and the resulting residue cooled (ice), and treated carefully with saturated NaHCO $_3$  (65 mL). The mixture was diluted with EtOAc (65 mL), and after 16 h stirring at room temperature the inorganic precipitate was removed by filtration through celite. The organic layer was separated, washed with brine (100 mL), dried (MgSO $_4$ ), filtered and evaporated in vacuo to give the crude amine 21 as a pale orange glass (0.29 g, 88%) which was carried through to the next step without further purification or analysis due to the instability of the amine.

(2S) - N - [(2-Allyloxycarbonylamino) - 4-benzyloxy - 5-methoxybenzoyl] - 2-(hydroxymethyl) - 4-(methoxycarbonylmethyl) - 2,3-dihydropyrrole (22).

A solution of the amino-alcohol 21 (0.29 g, 0.68 mmol) in  $CH_2Cl_2$  (12 mL) was cooled to 0°C (ice/acetone) and treated with pyridine (0.11 mL, 0.11 g, 1.39 mmol). A solution of allyl chloroformate (79  $\mu$ L, 90 mg, 0.75 mmol) in  $CH_2Cl_2$  (10 mL) was then added dropwise to the stirred mixture. The reaction mixture was allowed to warm to room temperature and stirred for a further 2.5 h, at which point TLC (EtOAc) revealed complete consumption of

the amine 21. The mixture was diluted with  $CH_2Cl_2$  (30 mL) and washed with saturated  $CuSO_4$  (20 mL),  $H_2O$  (20 mL), brine (20 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo. The crude residue was purified by flash chromatography (70% EtOAc/Petroleum Ether) to afford the pure alloc-amino compound 22 as a colourless 5 glass (0.14 g, 40%):  ${}^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  8.58 (br s, 1H, NH), 7.88 (br s,  $1H_{arom}$ ), 7.50-7.29 (m,  $5H_{arom}$ ), 6.83 (s,  $1H_{arom}$ ), 6.42 (br s, 1H,  $NCH=CCH_2CO_2CH_3$ ), 6.03-5.89 (m, 1H,  $NCO_2CH_2CH=CH_2$ ), 5.39-5.22 (m, 2H,  $NCO_2CH_2CH=CH_2$ ), 5.18 (s, 2H,  $PhCH_2O$ ), 4.77-4.73 (m, 1H, NCHCH<sub>2</sub>OH), 4.65-4.62 (m, 2H, NCO<sub>2</sub>CH<sub>2</sub>CH=CH<sub>2</sub>), 4.32-3.8410 (m, 5H, NCHCH<sub>2</sub>OH and OCH<sub>3</sub>), 3.69 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 3.09 (s, 2H, $NCH=CCH_2CO_2CH_3$ ), 3.05-2.95 (m, 1H,  $NCH=CCH_2CO_2CH_3CH_2$ ), 2.35 (dd, 1H, J = 3.76, 16.72 Hz, NCH=CCH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>CH<sub>2</sub>); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  170.6 ( $CO_2CH_3$ ), 167.4 ( $NC=O_{amide}$ ), 153.5 ( $NC=O_{carbamate}$ ), 151.1  $(C_{arom})$ , 144.4  $(C_{arom})$ , 136.1  $(C_{arom})$ , 132.6  $(C_{arom})$ , 132.4 15  $(NCO_2CH_2CH=CH_2)$ , 128.6, 128.1 and 127.7  $(BnC-H_{arom})$ , 118.5  $(NCH=CCH_2CO_2CH_3)$ , 118.2  $(NCO_2CH_2CH=CH_2)$ , 112.1  $(C-H_{arom})$ , 106.3  $(C-H_{arom})$  $H_{arom}$ ), 70.7 (PhCH<sub>2</sub>O), 66.5 (NCHCH<sub>2</sub>OH), 65.9 (NCO<sub>2</sub>CH<sub>2</sub>CH=CH<sub>2</sub>), 61.9  $(NCHCH_2OH)$ , 56.7  $(OCH_3)$ , 52.1  $(CO_2CH_3)$ , 35.6  $(NCH=CCH_2CO_2CH_3)$ , 33.6 (NCH=CCH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>CH<sub>2</sub>); MS (FAB), m/z (relative intensity) 618 20  $(M^{+} + Thioglycerol, 2), 511 (M^{+} + 1, 5), 510 (M^{+}, 1), 340 (M^{-})$ NCH=CCH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>CH<sub>2</sub>CHCH<sub>2</sub>OH, 20), 300 (3), 282 (14), 256 (7), 192 (6), 171 (16), 149 (22), 140 (12), 112 (4), 91 (PhCH<sub>2</sub>, 100), 80 (6), 65 (1), 57 (3).

(11S,11aS)-10-Allyloxycarbonyl-8-benzyloxy-11-hydroxy-7-methoxy-2-(methoxycarbonylmethyl)-1,10,11,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (23).

A solution of the alcohol 22 (0.14 g, 0.28 mmol) in  $CH_2Cl_2/CH_3CN$ (12 mL, 3:1) was treated with 4  $\mbox{\normalfont\AA}$  powdered molecular sieves (0.15 5 g) and NMO (49 mg, 0.42 mmol). After 15 min stirring at room temperature, TPAP (4.90 mg, 14 µmol) was added and stirring continued for a further 1 h 30 min at which point TLC (80% EtOAc/Petroleum Ether) showed product formation along with some unoxidised starting material. The mixture was then treated with a further quantity of NMO (49 mg, 0.42 mmol) and TPAP (4.9 mg, 14 umol), and allowed to stir for a further 0.5 h when TLC revealed reaction completion. The mixture was evaporated in vacuo onto silica and subjected to flash chromatography (60% EtOAc/Petroleum Ether) to provide the protected carbinolamine 23 as a colourless 15 glass (39 mg, 28%):  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.43-7.25 (m,  $6H_{arom}$ ), 6.90 (br s,  $1H_{arom}$ ), 6.74 (s, 1H,  $NCH=CCH_2CO_2CH_3$ ), 5.79-5.64 (m, 1H,  $NCO_2CH_2CH=CH_2$ ), 5.77 (d, 1H, J = 10.26 Hz, NCHCHOH), 5.19-5.06 (m, 4H,  $NCO_2CH_2CH=CH_2$  and  $PhCH_2O$ ), 4.64-4.45 (m, 2H,  $NCO_2CH_2CH=CH_2$ ), 4.18-3.83 (m, 4H, OCH<sub>3</sub> and NCHCHOH), 3.71 (s, 3H, 20  $CO_2CH_3$ ), 3.19 (s, 2H, NCH=CC $H_2CO_2CH_3$ ), 3.09 (dd, 1H, J = 11.09, 16.70 Hz, NCH=CCH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>CH<sub>2</sub>), 2.74 (d, 1H, J = 17.03 Hz,  $NCH=CCH_2CO_2CH_3CH_2$ ); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  170.7 ( $CO_2CH_3$ ), 163.3 ( $NC=O_{amide}$ ), 155.9 ( $NC=O_{carbamate}$ ), 150.3 ( $C_{arom}$ ), 149.1  $(C_{\text{arom}})$ , 136.1  $(C_{\text{arom}})$ , 131.8  $(NCO_2CH_2CH=CH_2)$ , 128.7, 128.2 and 25 127.3 (BnC- $H_{arom}$ ), 126.2 (NCH=CCH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>), 125.1 ( $C_{arom}$ ), 118.1  $(NCO_2CH_2CH=CH_2)$ , 117.7  $(NCH=CCH_2CO_2CH_3)$ , 114.7  $(C-H_{arom})$ , 111.0  $(C-H_{arom})$  $H_{arom}$ ), 85.9 (NCHCHOH), 71.1 (PhCH<sub>2</sub>O), 66.8 (NCO<sub>2</sub>CH<sub>2</sub>CH=CH<sub>2</sub>), 59.5

(NCHCHOH), 56.2 (OCH<sub>3</sub>), 52.1 (CO<sub>2</sub>CH<sub>3</sub>), 37.0 (NCH=CCH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>), 33.7 (NCH=CCH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>CH<sub>2</sub>); MS (EI), m/z (relative intensity) 508 (M<sup>+</sup>·, 16), 449 (3), 422 (3), 404 (2), 368 (3), 340 (19), 324 (2), 282 (6), 255 (2), 225 (1), 206 (2), 192 (3), 169 (4), 152 (2), 140 (10), 131 (5), 108 (5), 91 (PhCH<sub>2</sub>, 100), 80 (9), 57 (9); IR (NUJOL®) 3600-2500 (br, OH), 2924, 2853, 2360, 1715, 1602, 1514, 1462, 1377, 1271, 1219, 1169, 1045, 722, 699; exact mass calcd for  $C_{27}H_{28}N_2O_8$  m/e 508.1846, obsd m/e 508.1791.

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(11S,11aS) & (11R,11aS) -8-Benzyloxy-7,11-dimethoxy-2- (methoxycarbonylmethyl) -1,10,11,11a-tetrahydro-SH-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (25, SJG-245).

A catalytic amount of tetrakis(triphenylphosphine)palladium (5.0 mg, 4.33 µmol) was added to a stirred solution of the Allocprotected carbinolamine 23 (88 mg, 0.17 mmol), triphenylphosphine (2.27 mg, 8.65  $\mu$ mol) and pyrrolidine (13 mg, 0.18 mmol) in  $CH_2Cl_2$ 15 (15 mL). After 2 h stirring at room temperature under a nitrogen atmosphere, TLC (80% EtOAc/Petroleum Ether) revealed the complete consumption of starting material. The solvent was evaporated in vacuo and the crude residue was purified by flash chromatography (60% EtOAc/Petroleum Ether) to afford the novel PBD (SJG-245) as 20 a colourless glass (54 mg, 77%) which was repeatedly evaporated in vacuo with CHCl3 in order to provide the N10-C11 imine form 24:  $^{1}\text{H}$  NMR (270 MHz, CDCl<sub>3</sub>) (imine)  $\delta$  7.80 (d, 1H, J = 4.03 Hz, HC=N), 7.50 (s,  $1H_{arom}$ ), 7.45-7.26 (m,  $5H_{arom}$ ), 6.91 (br s, 1H,  $NCH=CCH_2CO_2CH_3$ ), 6.83 (s,  $1H_{arom}$ ), 5.21-5.12 (m, 2H,  $PhCH_2O$ ), 3.94 (s, 3H,  $OCH_3$ ), 3.73 (s, 3H,  $CO_2CH_3$ ), 3.23 (s, 2H,  $NCH=CCH_2CO_2CH_3$ ), 3.15 (m, 2H, NCH=CCH $_2$ CO $_2$ CH $_3$ CH $_2$ );  $^{13}$ C NMR (67.8 MHz, CDCl $_3$ ) (imine)

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Repeated evaporation in vacuo of 24 with CH3OH provided the N10-C11 methyl ether forms 25:  $^{1}\mathrm{H}$  NMR (270 MHz, CD $_{3}\mathrm{OD}$ ) (11S,11aSisomer)  $\delta$  7.44-7.25 (m,  $5H_{arom}$ ), 7.16 (s,  $1H_{arom}$ ), 6.85 (br s, 1H,  $NCH=CCH_2CO_2CH_3$ ), 6.62 (s,  $1H_{arom}$ ), 5.09 (s, 2H,  $PhCH_2O$ ), 4.52 (d, 10 1H, J = 8.80 Hz, NCHCHOCH<sub>3</sub>), 4.00-3.85 (m, 1H, NCHCHOCH<sub>3</sub>), 3.80 (s, 3H, OC $H_3$ ), 3.69 (s, 3H, CO<sub>2</sub>C $H_3$ ), 3.41 (s, 3H, NCHCHOC $H_3$ ), 3.24 (br s, 2H, NCH= $CCH_2CO_2CH_3$ ), 3.20-3.00 (m, 1H,  $NCH = CCH_2CO_2CH_3CH_2$ ), 2.60-2.50 (m, 1H,  $NCH = CCH_2CO_2CH_3CH_2$ ); <sup>13</sup>C NMR (67.8 MHz, CD<sub>3</sub>OD) (11S,11aS isomer)  $\delta$  172.7 ( $CO_2CH_3$ ), 166.8 15  $(C_{arom})$ , 153.3 (NC=O), 146.4  $(C_{arom})$ , 139.7  $(C_{arom})$ , 138.0  $(C_{arom})$ , 132.4 ( $C_{arom}$ ), 129.6, 129.1 and 128.8 (BnC-H<sub>arom</sub>), 127.0  $(NCH=CCH_2CO_2CH_3)$ , 120.8  $(NCH=CCH_2CO_2CH_3)$ , 113.7  $(C-H_{arom})$ , 109.2  $(C-H_{arom})$ , 97.1 (NCHCHOCH<sub>3</sub>), 71.7 (PhCH<sub>2</sub>O), 60.2 (NCHCHOCH<sub>3</sub>), 56.8  $(OCH_3)$ , 55.2  $(NCHCHOCH_3)$ , 52.5  $(CO_2CH_3)$ , 38.7  $(NCH=CCH_2CO_2CH_3)$ , 20 34.1 (NCH=CCH $_2$ CO $_2$ CH $_3$ CH $_2$ ); MS (EI), m/z (relative intensity) 420  $(M^+, methyl ether, 1)$ , 418 (methyl ether - 2, 2), 406  $(M^+, methyl ether)$ imine, 23), 404 (41), 375 (2), 345 (6), 333 (7), 313 (22), 299 (10), 285 (6), 253 (6), 242 (4), 225 (2), 214 (2), 198 (2), 183 (4), 168 (2), 155 (6), 136 (3), 105 (3), 91 (PhCH<sub>2</sub>, 100), 80 (4), 25 65 (7); IR (NUJOL $^{\otimes}$ ) 3318 (br, OH of carbinolamine form), 2923, 2853, 1737, 1692, 1658, 1627, 1601, 1552, 1511, 1501, 1464, 1461, 1452, 1378, 1244, 1072, 1006, 786, 754, 698 cm $^{-1}$ ; exact mass calcd for  $C_{23}H_{22}N_2O_5$  m/e 406.1529, obsd m/e 406.1510.

# Examples 1(c&d): Synthesis of SJG-301 (UP2051) and SJG-303 (UP2052) (see Figure 3)

Example 1(c)

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Example 1(d)

(2S)-N-[(2-Allyloxycarbonylamino)-4-benzyloxy-5-methoxybenzoyl]2-(tert-butyldimethylsilyloxymethyl)-4-(methoxycarbonylmethyl)2,3-dihydropyrrole (26)

Petroleum Ether (100 mL) was added to a sample of NaH (1.41 g of a 60% dispersion in oil, 35.25 mmol) and stirred at room temperature under a nitrogen atmosphere. After 0.5 h the mixture was allowed to settle and the Petroleum Ether was transferred from the flask via a double-tipped needle under nitrogen. THF (80 mL) was added to the remaining residue and the mixture was cooled to 0 °C (ice/acetone). The cool solution was treated dropwise with a solution of methyldiethylphosphonoacetate (6.47 mL, 7.41 g, 35.25 mmol) in THF (80 mL) under nitrogen. After 1.5 h at room temperature, the mixture was cooled to 0°C and treated dropwise with a solution of the ketone 6 (8.0 g, 14.1 mmol) in THF (50 mL) under nitrogen. After 16 h at room temperature, TLC (20% EtOAc/Petroleum Ether) revealed reaction

completion. The THF was evaporated in vacuo and the mixture partitioned between saturated NaHCO3 (200 mL) and EtOAc (220 mL). The layers were separated and the aqueous layer extracted with EtOAc (2 X 200 mL). The combined organic layers were washed with  $\rm H_2O$  (200 mL), brine (200 mL), dried (MgSO<sub>4</sub>), filtered and 5 concentrated in vacuo to give a dark red oil. Purification by flash chromatography (15% EtOAc/Petroleum Ether) furnished the endo-ester **26** (7.02 g, 80%):  $[\alpha]^{22}_{D} = -93.0$  ° (c = 1.04, CHCl<sub>3</sub>);  $^{1}\text{H}$  NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  8.78 (br s, 1H), 7.95 (s, 1H), 7.50-7.29 (m, 5H), 6.82 (s, 1H), 6.46 (br s, 1H), 6.02-5.88 (m, 1H), 10 5.35 (dd, 1H, J = 2.93, 17.22 Hz), 5.24 (d, 1H, J = 10.44 Hz), 5.18 (s, 2H), 4.70-4.61 (m, 3H), 3.96-3.82 (m, 5H), 3.68 (s, 3H), 3.08 (s, 2H), 2.91-2.82 (m, 1H), 2.71-2.65 (m, 1H), 0.88 (s, 9H), 0.06 and 0.04 (s x 2, 6H);  $^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  170.7, 165.8, 153.5, 150.6, 144.0, 136.2, 132.7, 132.5, 128.6, 128.2, 15 128.1, 127.7, 118.1, 118.0, 114.4, 112.0, 106.0, 70.6, 65.7, 62.3, 59.4, 56.6, 52.0, 34.6, 33.9, 25.8, 18.1, -5.4; MS (EI), m/z (relative intensity) 626 (M<sup>+</sup>· + 1, 3), 625 (M<sup>+</sup>· + 1, 7), 624  $(M^+, 14), 568 (5), 567 (11), 509 (3), 476 (3), 341 (5), 340 ...$ (17), 339 (4), 299 (3), 286 (18), 285 (87), 282 (11), 256 (4), 20 242 (3), 229 (3), 228 (14), 226 (11), 168 (10), 166 (3), 152 (6), 141 (5), 140 (50), 139 (9), 108 (3), 92 (10), 91 (100), 89 (6), 80 (11), 75 (11), 73 (10), 65 (5), 57 (6), 41 (12); IR (NEAT) 3332 (br, NH), 3019, 2953, 2930, 2857, 1733, 1622, 1599, 1524, 1491, 1464, 1408, 1362, 1335, 1258, 1205, 1171, 1113, 1051, 1027, 25 938, 839, 757, 697, 666 cm $^{-1}$ ; exact mass calcd for  $C_{33}H_{44}N_2O_8Si$ m/e 624.2867, obsd m/e 624.2936.

(2S) -N-[(2-Allyloxycarbonylamino)-4-benzyloxy-5-methoxybenzoyl]-2-(tert-butyldimethylsilyloxymethyl)-4-(hydroxy-2-ethyl)-2,3-dihydropyrrole (27)

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A solution of the ester 26 (4.0 g, 6.41 mmol) in THF (55 mL) was cooled to  $0^{\circ}$ C (ice/acetone) and treated with LiBH<sub>4</sub> (0.21 g, 9.62 mmol) in portions. The mixture was allowed to warm to room temperature and stirred under a nitrogen atmosphere for 26 h at which point TLC (50% EtOAc/Petroleum Ether) revealed the complete consumption of starting material. The mixture was cooled to 0°C (ice/acetone) and water (14 mL) was carefully added. Following evaporation of the THF in vacuo, the mixture was cooled and then neutralised with 1 N HCl. The solution was then diluted with  ${\rm H}_2{\rm O}$ (100 mL) and extracted with EtOAc (3 X 100 mL), the combined organic layers washed with brine (100 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo. The crude oil was purified by flash chromatography (30 - 40% EtOAc/Petroleum Ether) to furnish the pure endo-alcohol 27 as a transparent yellow oil (2.11 g, 55%):  $[\alpha]^{22}_{D} = -86.43$  ° (c = 1.38, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  8.76 (br s, 1H), 7.92 (br s, 1H), 7.50-7.28 (m, 5H), 6.82 (s, 1H), 6.36 (br s, 1H), 6.02-5.87 (m, 1H), 5.35 (d, 1H, J = 17.22Hz), 5.24 (d, 1H, J = 11.72 Hz), 5.18 (s, 2H), 4.64-4.61 (m, 3H), 4.10-3.99 (m, 1H), 3.80 (s, 3H), 3.79-3.66 (m, 3H), 2.85-2.75 (m, 1H), 2.64-2.60 (m, 1H), 2.30 (t, 2H, J = 6.23 Hz), 1.74 (br s, 1H), 0.88 (s, 9H), 0.06 and 0.04 (s x 2, 6H);  $^{13}$ C NMR (67.8 MHz,  $CDCl_3$ )  $\delta$  165.3, 153.5, 150.5, 144.2, 136.3, 132.5, 128.6, 128.1, 127.7, 126.7, 122.8, 118.0, 114.3, 112.0, 106.1, 70.7, 65.7, 62.8, 60.4, 59.1, 56.6, 34.4, 31.7, 25.8, 18.2, -5.4; MS (EI), m/z (relative intensity) 598 (M<sup>+.</sup> + 2, 3), 597 (M<sup>+.</sup> + 1, 5), 596

(M<sup>+</sup>·, 13), 581 (2), 541 (2), 540 (4), 539 (9), 448 (2), 341 (2), 340 (12), 282 (7), 259 (5), 258 (20), 257 (100), 256 (3), 227 (3), 226 (12), 200 (5), 168 (6), 124 (3), 113 (3), 112 (50), 111 (4), 94 (10), 91 (25), 73 (3); IR (NEAT) 3340 (br), 3066, 3033, 2930, 2857, 1732, 1598, 1520, 1456, 1409, 1328, 1205, 1166, 1113, 1049, 1023, 938, 839, 778, 744, 697, 677, 637 cm<sup>-1</sup>.

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(2S)-N-[(2-Allyloxycarbonylamino)-4-benzyloxy-5-methoxybenzoyl]-4-(acyloxy-2-ethyl)-2-(tert-butyldimethylsilyloxymethyl)-2,3-dihydropyrrole (28)

Acetic anhydride (8.17 g, 7.55 mL, 80 mmol) and pyridine 10 (30.2 mL) were added to the alcohol 27 (0.953 g, 1.60 mmol) and the solution stirred for 16 h under nitrogen at which point TLC revealed reaction completion (50% EtOAc/Petroleum Ether). The reaction mixture was cooled to 0°C (ice/acetone) and treated dropwise with MeOH (15 mL). After stirring at room temperature 15 for 1 h the mixture was treated dropwise with  $H_2O$  (30.2 mL) and allowed to stir for a further 16 h. Following dilution with EtOAc (56 mL), the solution was cooled to 0°C and treated dropwise with 6 N HCl (56 mL). The layers were separated and the organic phase was washed with 6N HCl (2 X 28 mL) and the combined 20 aqueous layers were then extracted with EtOAc (70 mL). combined organic phases were then washed with H2O (60 mL), brine (60 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo. crude oil was a mixture of the desired product 28 and the TBDMS cleaved compound 29 as judged by TLC. Purification by flash 25 chromatography (20 - 100% EtOAc/Petroleum Ether) provided 29 (0.2 g) and desired acyl-TBDMS compound 28 (0.59 g, 58%) as a

colourless oil:  $[\alpha]^{22}_D = -87.04$  ° (c = 4.91, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) (Rotamers) δ 8.77 (br s, 1H), 7.94 (br s, 1H), 7.49-7.31 (m, 5H), 6.80 (s, 1H), 6.37 (br s, 1H), 6.02-5.89 (m, 1H), 5.35 (dd, 1H, J = 17.22, 1.65 Hz), 5.24 (d, 1H, J = 10.30 Hz), (\*) 5.19 (s, 2H), 4.64-4.61 (m, 3H), 4.12 (t, 2H, J = 6.78 Hz), 4.03-5 3.95 (m, 1H), 3.83-3.75 (m, 4H), 2.85-2.75 (m, 1H), 2.64-2.60 (m, 1H), 2.40-2.26 (m, 2H,), 2.03 (s, 3H), 0.88 (s, 9H), 0.04, 0.01 and -0.01(s x 3, 6H);  $^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  170.9, 165.5, 153.5, 150.6, 144.1, 136.3, 132.7, 132.5, 128.6, 128.1, 127.7, 126.5, 122.2, 118.0, 114.3, 112.2, 106.1, 70.7, 65.7, 62.4, 60.4, 10 59.2, 56.7, 34.6, 31.7, 27.9, 25.8, 20.9, 18.2, -5.4; MS (EI), m/z (relative intensity) 640 (M<sup>+</sup>· + 2, 3), 639 (M<sup>+</sup>· + 1, 7), 638  $(M^+, 15), 623$  (2), 583 (3), 582 (6), 581 (14), 539 (2), 523 (3), 490 (3), 341 (5), 340 (22), 301 (5), 300 (18), 299 (75), 283 (3), 282 (14), 256 (4), 242 (7), 241 (5), 240 (16), 239 (62), 226 (6), 15 192 (3), 182 (8), 181 (5), 180 (3), 168 (5), 166 (5), 154 (10), 131 (3), 106 (3), 95 (4), 94 (48), 93 (5), 92 (8), 91 (100), 89 (5), 75 (6), 73 (8), 65 (3), 57 (3); IR (NEAT) 3324 (br, NH), 3066, 3018, 2954, 2930, 2857, 1737, 1622, 1598, 1523, 1489, 1464, 1409, 1363, 1327, 1230, 1205, 1168, 1115, 1080, 1030, 994, 937, 20 839, 756, 697, 667, 638, 606, 472, 459, 443  $\rm cm^{-1}$ ; exact mass

(2S)-N-[(2-Allyloxycarbonylamino)-4-benzyloxy-5-methoxybenzoyl]-4-(acyloxy-2-ethyl)-2-(hydroxymethyl)-2,3-dihydropyrrole (29)

A solution of the silyl ether 28 (0.83 g, 1.30 mmol) in THF (14 mL) was treated with  $\rm H_2O$  (14 mL) and glacial acetic acid (42 mL). After 2 h stirring at room temperature TLC (50%

calcd for  $C_{34}H_{46}N_2O_8Si$  m/e 638.3024, obsd m/e 638.3223.

EtOAc/Petroleum Ether) showed the complete consumption of starting material. The mixture was cooled (ice) and treated dropwise with a solution of  $NaHCO_3$  (64 g) in  $H_2O$  (640 mL). aqueous solution was extracted with EtOAc (3 X 100 mL) and the combined organic layers were washed with  ${\rm H}_2{\rm O}$  (150 mL), brine 5 (100 mL), dried (MgSO $_4$ ), filtered and concentrated in vacuo to give the crude product as an orange oil. Purification by flash chromatography (60% EtOAc/Petroleum Ether) furnished the pure alcohol **29** as a white glass (0.537 g, 81%):  $[\alpha]^{21}_{D} = -83.60$  ° (c = 0.25, CHCl<sub>3</sub>);  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  8.56 (br s, 1H), 7.89 10 (br s, 1H), 7.49-7.29 (m, 5H), 6.81 (s, 1H), 6.28 (br s, 1H), 6.03-5.89 (m, 1H), 5.35 (ddd, 1H, J = 17.22, 3.11, 1.46, Hz), 5.25 (d, 1H, J = 10.44 Hz), 5.19 (s, 2H), 4.80-4.70 (m, 1H), 4.65-4.62 (m, 2H), 4.41-4.31 (m, 1H), 4.20-4.06 (m, 2H,), 3.84-3.77 (m, 5H), 2.98-2.88 (m, 1H), 2.39 (t, 2H, J=6.51 Hz), 2.33-15 2.25 (m, 1H,), 2.03 (s, 3H);  $^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  170.8, 167.1, 153.5, 151.0, 144.3, 136.1, 132.6, 132.4, 128.6, 128.1, 127.7, 126.3, 122.6, 118.1, 112.2, 106.3, 70.7, 66.5, 65.8, 62.0, 61.7, 56.8, 35.4, 31.7, 27.8, 20.9; MS (EI), m/z (relative intensity) 525  $(M^{+} + 1, 5)$ , 524  $(M^{+}, 14)$ , 341 (5), 340 (16), 20 299 (2), 283 (3), 282 (14), 256 (4), 227 (5), 208 (2), 192 (3), 190 (2), 186 (9), 185 (60), 168 (2), 167 (5), 166 (2), 164 (2), 163 (2), 154 (3), 136 (3), 131 (3), 126 (7), 125 (53), 108 (2), 107 (2), 106 (2), 105 (3), 95 (3), 94 (19), 93 (3), 92 (9), 91 (100), 83 (2), 69 (2), 68 (3), 67 (3), 65 (5), 58 (6), 57 (17); 25 IR (CHCl<sub>3</sub>) 3335 (br), 2933, 1732, 1599, 1524, 1455, 1434, 1408, 1231, 1170, 1112, 1029, 995, 932, 868, 765, 698, 638, 606  $\text{cm}^{-1}$ ; exact mass calcd for  $C_{28}H_{32}N_2O_8$  m/e 524.2159, obsd m/e 524.2074.

(11S,11aS)-2-(Acyloxy-2-ethyl)-10-allyloxycarbonyl-8-benzyloxy11-hydroxy-7-methoxy-1,10,11,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (30)

Method A: A solution of DMSO (0.25 mL, 0.27 g, 3.49 mmol) in  $\mathrm{CH_2Cl_2}$  (10 mL) was added dropwise over 35 min to a solution of 5 oxalyl chloride (0.87 mL of a 2.0 M solution in  $CH_2Cl_2$ , 1.75 mmol) at -45°C (liq. $N_2$ /Chlorobenzene) under a nitrogen atmosphere. After stirring at -45°C for 40 min, a solution of the alcohol 29 (0.51 g, 0.97 mmol) in  $\mathrm{CH_2Cl_2}$  (7 mL) was added dropwise over 35 min at -45°C. After 55 min at -45°C, the 10 mixture was treated dropwise with a solution of TEA (0.57 mL, 0.41 g, 4.10 mmol) in  $CH_2Cl_2$  (5 mL) over 40 min at -45°C. After a further 45 min, the reaction mixture was allowed to warm to room temperature and was diluted with  $\mathrm{CH_2Cl_2}$  (60 mL), washed with 1N HCl (60 mL),  $H_2O$  (60 mL), brine (30 mL), dried (MgSO<sub>4</sub>), 15 filtered and evaporated in vacuo. TLC (80% EtOAc/Petroleum Ether) of the crude material revealed complete reaction. Purification by flash chromatography (50% EtOAc/Petroleum Ether) furnished the protected carbinolamine 30 as a creamy glass (0.25 g, 49%). 20

Method B: A solution of the alcohol 29 (0.21 g, 0.40 mmol) in  $CH_2Cl_2/CH_3CN$  (30 mL, 3:1) was treated with 4 Å powdered molecular sieves (0.15 g) and NMO (69 mg, 0.59 mmol). After 15 min stirring at room temperature, TPAP (6.9 mg, 19.8 µmol) was added and stirring continued for a further 1 h at which point TLC (80% EtoAc/Petroleum Ether) showed product formation along with some unoxidised starting material. The mixture was then treated with a further quantity of NMO (35 mg, 0.30 mmol) and TPAP (3.50 mg,

25

10 µmol), and allowed to stir for a further 1.5 h after which time TLC revealed complete reaction. The mixture was evaporated in vacuo onto silica and subjected to flash chromatography (50% EtOAc/Petroleum Ether) to provide the protected carbinolamine 30 as a creamy glass (95 mg, 46%):  $[\alpha]^{20}_{D} = +113.85$  ° (c = 0.95, 5  $CHCl_2$ ); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.49-7.26 (m, 6H), 6.80 (s, 1H), 6.76 (s, 1H), 5.79-5.59 (m, 1H), 5.75 (d, 1H, J = 10.08 Hz), 5.19-5.05 (m, 4H), 4.52-4.29 (m, 2H), 4.28-4.08 (m, 3H), 3.95-3.80 (m, 4H), 2.99 (dd, 1H, J = 10.72, 16.94 Hz), 2.66 (d, 1H, J= 16.86 Hz), 2.46 (t, 2H, J = 6.41 Hz), 2.06 (s, 3H); <sup>13</sup>C NMR 10 (67.8 MHz, CDCl<sub>3</sub>) δ 171.1, 163.1, 155.9, 150.3, 149.1, 136.1, 131.8, 128.7, 128.6, 128.2, 127.3, 125.3, 124.4, 121.6, 118.0, 114.8, 111.0, 85.9, 71.1, 66.8, 62.0, 70.7, 59.4, 56.2, 37.0, 27.9, 21.0; MS (EI), m/z (relative intensity) 522 (M<sup>+</sup>·, 13), 463 (9), 462 (13), 341 (8), 340 (32), 282 (11), 256 (3), 183 (5), 154 . 15 (3), 123 (8), 94 (20), 91 (100), 65 (4), 57 (15); exact mass calcd for  $C_{28}H_{30}N_2O_8$  m/e 522.2002, obsd m/e 522.2008.

Example 1(c): (11aS)-2-(Acyloxy-2-ethyl)-8-benzyloxy-7-methoxy-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (31,UP2051, SJG-301)

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A catalytic amount of tetrakis(triphenylphosphine)palladium (5.26 mg, 4.55 μmol) was added to a stirred solution of the Alloc-protected carbinolamine 30 (95 mg, 0.18 mmol), triphenylphosphine (2.39 mg, 9.10 μmol) and pyrrolidine (13.6 mg, 0.19 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL). After 1 h stirring at room temperature under a nitrogen atmosphere, TLC (97% CHCl<sub>3</sub>/MeOH) revealed the complete consumption of starting material. The solvent was evaporated in

vacuo and the crude residue was purified by flash chromatography (99.5%  $CHCl_3/MeOH$ ) to afford the PBD (31, SJG-301, UP2051) as an orange glass which was repeatedly evaporated in vacuo with  $CHCl_3$ in order to provide the N10-C11 imine form (66.3 mg, 87%):  $[\alpha]^{21}$ <sub>D</sub> = +741.67 ° (c = 0.66, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) (imine)  $\delta$ 5 7.78 (d, 1H, J = 4.03 Hz), 7.70-7.28 (m, 6H), 6.83 (s, 1H), 6.82 (s, 1H), 5.19-5.18 (m, 2H), 4.27-4.16 (m, 2H), 3.94 (s, 3H),3.44-3.35 (m, 1H), 3.28-3.15 (m, 1H), 3.04-2.97 (m, 1H), 2.52- $2.47 \text{ (m, 2H), } 2.06 \text{ (s, 3H); } ^{13}\text{C NMR (67.8 MHz, CDCl}_3) \text{ (Rotamers)}$ δ 170.9, 162.6, 161.1, 150.9, 148.2, 140.1, 136.1, 132.1, 132.0, 10 128.7, 128.6, 128.1, 127.3, 124.7, 121.4, 111.9, 111.6, 70.8, 61.9, 56.2, 53.6, 37.4, 27.9, 21.0; MS (EI), m/z (relative intensity) 421  $(M^{+} + 1, 4)$ , 420  $(M^{+}, 14)$ , 419 (12), 418 (36), 361 (6), 360 (20), 328 (3), 313 (8), 270 (4), 269 (7), 268 (9), 267 (22), 256 (4), 129 (3), 105 (3), 94 (4), 93 (3), 92 (12), 91 15 (100), 83 (3), 80 (3), 73 (5), 71 (3), 69 (3), 65 (5), 60 (4), 57 (5), 55 (4); IR (CHCl<sub>3</sub>) 3313 (br), 2957, 2934, 1736, 1598, 1509, 1455, 1437, 1384, 1243, 1179, 1120, 1096, 1037, 753, 696, 666, 542 cm $^{-1}$ ; exact mass calcd for  $C_{24}H_{24}N_2O_5$  m/e 420.1685, obsd m/e 420.1750. 20

(115,11aS)-10-Allyloxycarbonyl-8-benzyloxy-11-hydroxy-2-(hydroxy-2-ethyl)-7-methoxy-1,10,11,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (32).

A solution of  $K_2CO_3$  (328 mg, 2.38 mmol) in  $H_2O$  (6 mL) was added dropwise to a stirred solution of the acyl compound **30** (0.248 g, 0.475 mmol) in  $CH_2Cl_2$  (3 mL) and MeOH (8 mL). After stirring for 16 h at room temperature TLC (EtOAc) revealed complete reaction.

The MeOH/CH2Cl2 was evaporated in vacuo to give a cloudy aqueous solution which was diluted with  $H_2O$  (30 mL) and extracted with EtOAc (3 X 30 mL). The combined organic layers were then washed with brine (30 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo to provide a creamy oil. Purification by flash 5 chromatography (97% CHCl3/MeOH) furnished the homoallylic alcohol 32 as a transparent colourless glass (178 mg, 78%):  $[\alpha]^{21}_{D} =$ +48.43 ° (c = 1.56, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.43-7.24 (m, 6H), 6.84 (s, 1H), 6.73 (s, 1H), 5.74-5.55 (m, 1H), 5.73 (d,1H, J = 8.79 Hz), 5.19-5.06 (m, 4H), 4.46-4.23 (m, 2H), 3.92-3.70 10 (m, 6H), 3.07-2.97 (m, 1H), 2.67 (d, 1H), J = 16.49 Hz), 2.40-2.17(m, 2H);  $^{13}C$  NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  163.1, 155.8, 150.3, 149.1, 136.1, 131.8, 128.6, 128.1, 127.7, 127.4, 125.3, 124.1, 124.0, 123.1, 123.0, 117.9, 114.9, 110.9, 86.0, 71.1, 66.7, 60.3, 59.6, 56.2, 37.1, 31.5; MS (EI), m/z (relative intensity) 482 (M<sup>+.</sup> + 2, 15 4), 481  $(M^{+} + 1, 10)$ , 480  $(M^{+}, 26)$ , 449 (4), 378 (12), 347 (7), 341 (7), 340 (25), 339 (4), 284 (4), 282 (10), 143 (4), 141 (13), 131 (6), 112 (24), 110 (4), 94 (10), 92 (9), 91 (100), 80 (4), 70 (5), 69 (7), 65 (4), 58 (11), 57 (29); exact mass calcd for  $C_{26}H_{28}N_2O_7$  m/e 480.1897, obsd m/e 480.1886. 20

Example 1(d): (11aS)-8-Benzyloxy-2-(hydroxy-2-ethyl)-7-methoxy-1,2,3,11a-tetrahydro-<math>5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (33, UP2052, SJG-303).

A catalytic amount of tetrakis(triphenylphosphine)palladium (9.39 mg, 8.13 µmol) was added to a stirred solution of the Alloc-protected carbinolamine 30 (156 mg, 0.33 mmol), triphenylphosphine (4.26 mg, 16.3 µmol) and pyrrolidine (24.3 mg,

0.34 mmol) in  $\mathrm{CH_2Cl_2}$  (15 mL). After 1 h 50 min stirring at room temperature under a nitrogen atmosphere, TLC (90% CHCl<sub>3</sub>/MeOH) revealed the complete consumption of starting material. The solvent was evaporated in vacuo and the crude residue was purified by flash chromatography (98%  $CHCl_3/MeOH$ ) to afford the PBD (33, SJG-303, UP2052) as an orange glass which was repeatedly evaporated in vacuo with  $CHCl_3$  in order to provide the N10-C11imine form (103 mg, 84%):  $^{1}\text{H}$  NMR (270 MHz, CDCl $_{3}$ ) (Rotamers)  $\delta$ 7.75 (d, 1H, J = 4.03 Hz), 7.58-7.22 (m, 6H), 6.82-6.80 (m, 2H), 5.17-4.88 (m, 2H), 4.65-4.20 (m, 2H), 3.91 (s, 3H), 3.35-3.25 (m, 10 1H), 3.18-3.15 (m, 1H), 3.04-2.97 (m, 1H), 2.52-2.47 (m, 2H);  $^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>) (Rotamers)  $\delta$  162.8, 161.1, 152.3, 150.9, 148.1, 142.3, 138.3, 136.4, 128.7, 128.6, 128.2, 127.4, 124.2, 123.1, 111.8, 111.6, 70.8, 60.4, 56.2, 53.6, 37.7, 31.5; MS (EI), m/z (relative intensity) 380 (13), 379 (11), 378 (M<sup>+</sup>·, 42), 377 15 (36), 376 (77), 375 (6), 347 (8), 345 (5), 334 (5), 333 (19), 288 (14), 287 (14), 286 (36), 285 (50), 272 (6), 271 (22), 269 (6), 268 (6), 267 (5), 259 (5), 257 (13), 255 (24), 243 (15), 155 (6), 136 (5), 124 (7), 106 (6), 93 (6), 92 (38), 91 (100), 65 (16), 63 (5), 51 (5); IR (CHCl<sub>3</sub>) 3313, 2918, 1623, 1598, 1568, 1509, 1455, 20 1436, 1386, 1328, 1243, 1218, 1175, 1130 1061, 1007, 870, 831, 792, 752, 697, 662 cm $^{-1}$ ; exact mass calculated for  $C_{22}H_{22}N_2O_4$  m/e 378.1580, obsd m/e 378.1576.

Repeated evaporation in vacuo of UP2052 with CH<sub>3</sub>OH provided the N10-C11 methyl ether forms:  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>) (Rotamers)  $^{5}$  7.66-7.22 (m, 6H), 6.82-6.81 (m, 2H), 5.21-4.76 (m, 2H), 4.61-4.15 (m, 1H), 4.03-3.71 (m, 5H), 3.44 (s, 3H), 3.35-1.92 (m, 7H).

## Example 1(e):Synthesis of the C7,C8-Dimethoxy-C2Methoxycarbonylmethyl PBD AN-SJG (UP2065) (see Figure 4)

## (2S) (4R) -N-(4,5-Dimethoxy-2-nitrobenzoy1)-2-(tert-butyldimethylsilyloxymethyl)-4-hydroxypyrrolidine (35)

A catalytic amount of DMF (2 drops) was added to a stirred 5 solution of the nitro-acid 34 (12.45 g, 54.8 mmol) and oxalyl chloride (5.75 mL, 8.37 g, 65.9 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (300 mL). After 16 h at room temperature the resulting acid chloride solution was added dropwise over 4.5 h to a stirred mixture of the amine 2 (12.65 g, 54.8 mmol) and TEA (13.86 g, 19.1 mL, 137 mmol) in 10  $CH_2Cl_2$  (300 mL) at 0°C (ice/acetone) under a nitrogen atmosphere. The reaction mixture was allowed to warm to room temperature and stirred for a further 2.5 h. The mixture was washed with saturated NaHCO<sub>3</sub> (300 mL), saturated NH<sub>4</sub>Cl (300 mL),  $\rm H_2O$ (250 mL), brine (300 mL), dried (MgSO<sub>4</sub>), filtered and evaporated 15 in vacuo to give the crude product as a dark orange oil. Purification by flash chromatography (80% EtOAc/Petroleum Ether) isolated the pure amide 35 as a sticky orange oil (18.11 g, 75%):  $[\alpha]^{22}_{D} = -105.7^{\circ} (c = 1.17, CHCl_{3}); ^{1}H NMR (270 MHz, CDCl_{3})$ (Rotamers)  $\delta$  7.71 and 7.68 (s x 2, 1H), 6.86 and 6.79 (s x 2, 20 1H), 4.50 and 4.38 (br s x 2, 2H), 4.13-4.10 (m, 1H), 3.98 (s, 3H), 3.94 (s, 3H), 3.78-3.74 (m, 1H), 3.35-3.27 (m, 1H), 3.07 (d, 1H, J = 11.17 Hz), 3.01-2.79 (br s, 1H), 2.35-2.26 (m, 1H), 2.11-

2.04 (m, 1H), 0.91 and 0.81 (s x 2, 9H), 0.10, 0.09, -0.07, and -0.10 (s x 4, 6H);  $^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>) (Rotamers)  $\delta$  166.6, 154.2 and 154.1, 149.3 and 148.9, 137.5, 128.0, 109.2, 107.1, 70.1 and 69.4, 64.7 and 62.5, 59.0 and 54.9, 57.3, 56.6, 56.5, 37.4 and 36.3, 25.9 and 25.7, 18.2, -5.4, -5.5 and -5.7; MS (EI), 5 m/z (relative intensity) 440 (M<sup>+</sup>·, 2), 426 (9), 386 (4), 385 (20), 384 (65), 383 (100), 367 (4), 320 (4), 308 (7), 295 (8), 286 (5), 211 (15), 210 (100), 194 (12), 180 (4), 165 (17), 164 (8), 137 (4), 136 (25), 121 (4), 93 (6), 91 (9), 82 (6), 75 (15), 73 (15), 59 (4), 57 (4); IR (NEAT) 3391 (br, OH), 3012, 2952, 10 2931, 2857, 1616, 1578, 1522, 1456, 1436, 1388, 1338, 1279, 1225, 1183, 1151, 1074, 1053, 1029, 1004, 939, 870, 836, 816, 785, 757, 668, 650, 620  $\text{cm}^{-1}$ ; exact mass calcd for  $C_{20}H_{32}N_2O_7\text{Si}$  m/e 440.1979, obsd m/e 440.1903.

(2S) (4R) - N - (2-Amino-4, 5-dimethoxybenzoyl) - 2 - (tert-15 butyldimethylsilyloxymethyl)-4-hydroxypyrrolidine (36) A solution of hydrazine (6.59 g, 6.40 mL, 205.5 mmol) in MeOH (110 mL) was added dropwise to a solution of the nitro-compound 35 (18.1 g, 41.1 mmol), over anti-bumping granules and Raney Ni (2.6 g) in MeOH (325 mL) and heated at reflux. After 1 h at 20 reflux TLC (95% CHCl3/MeOH) revealed some amine formation. reaction mixture was treated with further Raney Ni (2.6 g) and hydrazine (6.40 mL) in MeOH (50 mL) and was heated at reflux for an additional 30 min at which point TLC revealed reaction completion. The reaction mixture was then treated with 25 sufficient Raney Ni to decompose any remaining hydrazine and heated at reflux for a further 1.5 h. Following cooling to room

temperature the mixture was filtered through a sinter and the resulting filtrate evaporated in vacuo. The resulting residue was then treated with CH<sub>2</sub>Cl<sub>2</sub> (300 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo to provide the amine 36 as a green oil  $(16.03 \text{ g}, 95\%): [\alpha]^{22}_D = -116.32 \circ (c = 0.31, CHCl_3); ^1H NMR (270)$ 5 MHz, CDCl<sub>3</sub>) (Rotamers)  $\delta$  6.70 (s, 1H), 6.28 (s, 1H), 4.51-4.49 (m, 1H), 4.36-4.34 (m, 1H), 4.06-3.77 (m, 10H), 3.61-3.50 (m, 1H)3H), 2.23-2.21 (m, 1H), 2.01-1.98 (m, 1H), 0.89 (s, 9H), 0.04 (s, 6H);  $^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>) (Rotamers)  $\delta$  170.2, 151.5, 141.2, 10 140.5, 112.2, 112.0, 101.1, 70.4, 62.6, 59.0, 56.9, 56.6, 55.8, 35.7, 25.9 and 25.7, 18.2, -5.4 and -5.5; MS (EI), m/z (relative intensity) 412  $(M^+ + 2, 3)$ , 411  $(M^+ + 1, 10)$ , 410  $(M^+, 32)$ , 354 (6), 353 (23), 263 (3), 212 (5), 181 (11), 180 (100), 179 (3), 165 (3), 164 (6), 152 (10), 137 (4), 136 (4), 125 (5), 120 (3), 100 (3), 94 (6), 75 (9), 73 (7), 57 (3); IR (CHCl<sub>3</sub>) 3353 (br), 15 2953, 2930, 2857, 1623, 1594, 1558, 1517, 1464, 1435, 1404, 1260, 1234, 1215, 1175, 1119, 1060, 1005, 915, 836, 777, 755, 666 cm<sup>-1</sup>; exact mass calcd for  $C_{20}H_{34}N_2O_5Si$  m/e 410.2237, obsd m/e 410.2281.

(2S) (4R)-N-[(2-Allyloxycarbonylamino)-4,5-dimethoxybenzoy1]-2
(tert-butyldimethylsilyloxymethyl)-4-hydroxypyrrolidine (37)

A solution of the amine 36 (16.03 g, 39 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (450 mL)

was cooled to 0°C (ice/acetone) and treated with pyridine

(6.94 mL, 6.78 g, 85.8 mmol). A solution of allyl chloroformate

(4.35 mL, 4.94 g, 40.95 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (90 mL) was then added

dropwise to the stirred mixture. The reaction mixture was allowed to warm to room temperature and stirred for a further 1.5 h, at which point TLC (EtOAc) revealed complete consumption of

amine 36. The reaction mixture was washed with saturated CuSO<sub>4</sub> (300 mL),  $H_2O$  (300 mL), brine (300 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo. The crude residue was purified by flash chromatography (35% EtOAc/Petroleum Ether) to afford the pure alloc-amino compound 37 as a clear oil (16.78 g, 87%):  $[\alpha]_{D}^{23} = -$ 5 93.35 ° (c = 0.27, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) (Rotamers)  $\delta$ 8.93 (br s, 1H), 7.72 (s, 1H), 6.77 (s, 1H), 6.01-5.87 (m, 1H), 5.34 (dd, 1H, J = 17.22, 3.12 Hz), 5.23 (dd, 1H, J = 10.44, 1.29 Hz), 4.63-4.55 (m, 3H), 4.40-4.38 (m, 1H), 4.15-4.08 (m, 1H), 3.91 (s, 3H), 3.81 (s, 3H), 3.62-3.55 (m, 3H), 2.34-2.24 (m, 2H), 10 2.07-1.99 (m, 1H), 0.89 (s, 9H), 0.05 and 0.04 (s x 2, 6H);  $^{13}C$ NMR (67.8 MHz, CDCl<sub>3</sub>) (Rotamers) δ 169.5, 153.8, 150.9, 143.8, 132.5, 118.0, 115.9, 111.0, 104.6, 70.5, 65.8, 62.2, 59.0, 57.2, 56.2, 56.0, 35.7 and 31.1, 25.8, 18.1, -5.4 and -5.5; MS (EI), m/z (relative intensity) 496 (M<sup>+</sup>· + 2, 6), 495 (M<sup>+</sup>· + 1, 18), 494 15  $(M^+, 50), 439 (11), 438 (29), 437 (100), 380 (4), 379 (14), 337$ (13), 336(4), 265 (15), 264 (91), 263 (4), 258 (6), 224 (4), 223 (15), 220 (11), 212 (7), 208 (4), 207 (11), 206 (75), 192 (5), 180 (20), 179 (18), 174 (15), 172 (4), 164 (7), 156 (5), 152 (5), 150 (6), 136 (4), 99 (9), 86 (16), 75 (10), 73 (11), 57 (6); IR 20 (CHCl<sub>3</sub>) 3337 (br), 2952, 2930, 2857, 1733, 1600, 1522, 1458, 1420, 1399, 1327, 1288, 1261, 1229, 1203, 1165, 1121, 1039, 1004, 931, 836, 777, 668  $\text{cm}^{-1}$ ; exact mass calcd for  $\text{C}_{24}\text{H}_{38}\text{N}_2\text{O}_7\text{Si}$  m/e 494.2448, obsd m/e 494.2365.

25 (2S)-N-[(2-Allyloxycarbonylamino)-4,5-dimethoxybenzoyl]-2-(tert-butyldimethylsilyloxymethyl)-4-oxopyrrolidine (38)

A solution of DMSO (7.24 mL, 7.97 g, 102 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (150 mL)

.was added dropwise over 2 h to a solution of oxalyl chloride (25.5 mL of a 2.0 M solution in  $CH_2Cl_2$ , 51.0 mmol) at -60°C (lig.N2/CHCl3) under a nitrogen atmosphere. After stirring at -50 °C for 1 h, a solution of the alcohol 37 (16.75 g, 33.9 mmol) in  $CH_2Cl_2$  (250 mL) was added dropwise over a period of 2 h. After 1 h at -55°C, the mixture was treated dropwise with a solution of TEA (32.2 mL, 23.4 g, 231 mmol) in  $CH_2Cl_2$  (100 mL) and allowed to warm to room temperature. The reaction mixture was treated with brine (250 mL) and washed with cold 1N HCl (2 X 300 mL). TLC (50% EtOAc/Petroleum Ether) analysis of the CH<sub>2</sub>Cl<sub>2</sub> 10 layer revealed complete reaction. The layers were separated and the organic phase washed with  $H_2O$  (300 mL), brine (300 mL), dried (MgSO<sub>4</sub>), filtered and concentrated in vacuo to give the ketone 38 as an orange glass (16.37 g, 98%):  $[\alpha]^{21}_{D} = -9.96$  ° (c = 1.51,CHCl<sub>3</sub>);  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  8.69 (s, 1H), 7.82 (s, 1H), 15 6.75 (s, 1H), 6.01-5.89 (m, 1H), 5.36 (dd, 1H, J = 17.22, 3.11 Hz), 5.28-5.23 (m, 1H), 5.20-4.95 (m, 1H), 4.65-4.62 (m, 2H), 4.20-3.83 (m, 9H), 3.67-3.56 (m, 1H), 2.74 (dd, 1H, J = 17.86, 9.44 Hz), 2.52 (d, 1H, J = 17.95 Hz), 0.87 (s, 9H), 0.05 (s, 6H);  $^{13}\text{C}$  NMR (67.8 MHz, CDCl<sub>3</sub>) (Rotamers)  $\delta$  208.9, 169.1, 153.5, 20 151.3, 143.9, 132.4, 118.2, 114.4, 110.1, 104.6, 66.1, 65.8, 56.2, 56.0, 39.7, 25.6, 18.0, -5.7 and -5.8; MS (EI), m/z(relative intensity) 494 ( $M^+$  + 2, 6), 493 ( $M^+$  + 1, 16), 492  $(M^+, 43), 437 (8), 436 (22), 435 (74), 377 (11), 336 (6), 335$ (21), 334 (8), 294 (8), 265 (9), 264 (50), 250 (5), 223 (17), 220 25 (18), 208 (7), 207 (15), 206 (100), 192 (9), 180 (23), 179 (28), 172 (33), 171 (10), 164 (16), 155 (7), 152 (9), 150 (16), 136 (13), 115 (14), 108 (6), 88 (6), 75 (20), 73 (33), 59 (13), 58

(6), 57 (62), 56 (14); IR (NEAT) 3337 (br, NH), 3086, 3019, 2954, 2932, 2858, 1766, 1732, 1623, 1603, 1520, 1464, 1398, 1362, 1332, 1313, 1287, 1262, 1204, 1166, 1110, 1052, 1038, 1004, 938, 870, 838, 810, 756, 666, 621, 600 cm<sup>-1</sup>; exact mass calcd for  $C_{24}H_{36}N_2O_7Si$  m/e 492.2292, obsd m/e 492.2349.

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2S) -N-[(2-Allyloxycarbonylamino)-4,5-dimethoxybenzoyl]-2-(tert-butyldimethylsilyloxymethyl)-4-(methoxycarbonylmethyl)-2,3-dihydropyrrole (39)

Petroleum ether (70 mL) was added to a sample of NaH (0.41 g of a 60% dispersion in oil, 10.16 mmol) and stirred at room 10 temperature under a nitrogen atmosphere. After 0.5 h the mixture was allowed to settle and the Petroleum Ether was transferred from the flask via a double-tipped needle under nitrogen. (60 mL) was added to the remaining residue and the mixture was cooled to 0°C (ice/acetone). The cool solution was treated 15 dropwise with a solution of methyldiethylphosphonoacetate (1.86 mL, 2.14 g, 10.16 mmol) in THF (60 mL) under nitrogen. After 1.5 h at room temperature, the mixture was cooled to  $0^{\circ}\mathrm{C}$ and treated dropwise with a solution of the ketone 38 (2.0 g, 4.07 mmol) in THF (36 mL) under nitrogen. After 16 h at room 20 temperature, TLC (20% EtOAc/Petroleum Ether) revealed reaction completion. The THF was evaporated in vacuo and the mixture partitioned between saturated  $NaHCO_3$  (100 mL) and EtOAc (100 mL). The layers were separated and the aqueous layer extracted with EtOAc (2 X 100 mL). The combined organic layers were washed with 25  ${\rm H_{2}O}$  (100 mL), brine (100 mL), dried (MgSO<sub>4</sub>), filtered and concentrated in vacuo to give a dark red oil. Purification by

flash chromatography (15% EtOAc/Petroleum Ether) furnished the endo-ester 39 as a golden oil (1.63 g, 73%): 1H NMR (270 MHz, CDCl<sub>3</sub>) (Rotamers)  $\delta$  8.82 (br s, 1H), 7.86 (s, 1H), 6.79 (s, 1H), 6.46 (br s, 1H), 6.03-5.89 (m, 1H), 5.39-5.32 (m, 1H), 5.24 (dd, 1H, J = 10.44, 1.28 Hz), 4.70-4.59 (m, 3H), 3.99-3.61 (m, 11H), 5 3.08 (s, 2H), 2.91-2.82 (m, 1H), 2.75-2.66 (m, 1H), 0.92-0.79 (m, 9H), 0.12-0.03 (m, 6H);  ${}^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>) (Rotamers)  $\delta$ 170.7, 165.8, 153.5, 151.3, 143.7, 132.8, 132.5, 128.2, 118.1, 118.0, 117.9, 111.3, 104.3, 65.7, 62.3, 59.5 and 59.4, 56.4, 56.0, 52.0, 34.7, 33.9, 25.8, 18.1, -5.4; MS (EI), m/z (relative 10 intensity) 549  $(M^+ + 1, 7)$ , 548  $(M^+, 17)$ , 525 (13), 507 (14), 492 (6), 491 (18), 489 (8), 449 (7), 347 (11), 287 (6), 286 (20), 285 (82), 265 (10), 264 (51), 263 (9), 244 (9), 242 (7), 228 (19), 227 (8), 226 (18), 224 (6), 223 (22), 220 (12), 208 (6), 207 (18), 206 (100), 192 (7), 180 (18), 179 (21), 168 (16), 164 15 (10),152 (13), 150 (8), 141 (8), 140 (73), 139 (13), 136 (6), 108 (6), 89 (9), 80 (15), 75 (15), 73 (19), 57 (6); exact mass calcd for  $C_{27}H_{40}N_2O_8Si$  m/e 548.2554, obsd m/e 548.2560

(2s)-N-[(2-Allyloxycarbonylamino)-4,5-dimethoxybenzoyl]-2
(hydroxymethyl)-4-(methoxycarbonylmethyl)-2,3-dihydropyrrole (40).

A solution of the silyl ether 39 (1.63 g, 2.97 mmol) in THF

(12.6 mL) was treated with H<sub>2</sub>O (12.6 mL) and glacial acetic acid

(38 mL). After 2 h stirring at room temperature TLC (60%

EtOAc/Petroleum Ether) showed the complete consumption of

25 starting material. The mixture was cooled (ice) and treated

dropwise with a solution of NaHCO<sub>3</sub> (61.6 g) in H<sub>2</sub>O (616 mL). The

aqueous solution was extracted with EtOAc (3 x 150 mL) and the

combined organic layers were washed with  $H_2O$  (150 mL), brine (100 mL), dried (MgSO<sub>4</sub>), filtered and concentrated *in vacuo* to give the crude alcohol **40** as an orange oil (1.27 g, 98%): MS (EI), m/z (relative intensity) 435 (M<sup>+</sup>· + 1, 6),434 (M<sup>+</sup>·, 23), 347 (5), 317 (4), 281 (6), 265 (8), 264 (44), 263 (8), 224 (5), 223 (24), 222 (5), 220 (9) 207 (15), 206 (94), 192 (5), 180 (18), 179 (18), 172 (12), 171 (100), 164 (12), 152 (7), 150 (7), 141 (6), 140 (53), 136 (9), 112 (11), 108 (6), 80 (12), 69 (7); exact mass calcd for  $C_{21}H_{26}N_2O_8$  m/e 434.1689, obsd m/e 434.1606.

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10 (11s,11as)-10-Allyloxycarbonyl-7,8-dimethoxy-11-hydroxy-2(methoxycarbonylmethyl)-1,10,11,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (41)

A solution of DMSO (0.75 mL, 0.82 g, 10.5 mmol) in  $CH_2Cl_2$  (22 mL) was added dropwise over 1 h 20 min to a solution of oxalyl chloride (2.63 mL of a 2.0 M solution in  $CH_2Cl_2$ , 5.26 mmol) at -15 45 °C ( $liq.N_2$ /Chlorobenzene) under a nitrogen atmosphere. After stirring at -45°C for 1 h, a solution of the alcohol 40 (1.27 g, 2.92 mmol) in  $CH_2Cl_2$  (22 mL) was added dropwise over 1 h at -45°C. After 50 min at -45°C, the mixture was treated dropwise with a solution of TEA (1.71 mL, 1.24 g, 12.29 mmol) in  $CH_2Cl_2$ 20 (11 mL) over 30 min at -45°C. After a further 30 min, the reaction mixture was allowed to warm to room temperature and was diluted with  $CH_2Cl_2$  (20 mL), washed with 1N HCl (100 mL),  $H_2O$ (100 mL), brine (100 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo. TLC (80% EtOAc/Petroleum Ether) of the crude material 25 revealed reaction completion. Purification by flash chromatography (55% EtOAc/Petroleum Ether) furnished the

protected carbinolamine 41 as a white glass (0.68 g, 54%):  $[\alpha]^{22}$ = +219.78 ° (c = 0.12, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.23 (s, 1H), 6.91 (s, 1H), 6.70 (s, 1H), 5.90-5.80 (m, 2H), 5.17-5.13 (m, 2H), 4.70 (dd, 1H, J = 13.37, 5.31 Hz), 4.50-4.43 (m, 1H), 3.98-3.75 (m, 8H), 3.71 (s, 3H), 3.20-3.05 (m, 3H), 2.75 (d, 1H, J =5 17.04 Hz);  $^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  170.7, 163.3, 155.9, 151.1, 148.5, 131.7, 128.3, 126.2, 124.7, 118.1, 117.6, 112.6, 110.6, 86.0, 66.8, 59.4, 56.2, 52.1, 37.0, 33.7; MS (EI), m/z (relative intensity) 434 ( $M^+$  + 2, 6), 433 ( $M^+$  + 1, 21), 432  $(M^+, 74), 414$  (8), 373 (14), 329 (7), 293 (20), 292 (20), 265 10 (19), 264 (100), 263 (33), 248 (25), 224 (6), 223 (25), 220 (14), 209 (8), 208 (52), 207 (24), 206 (92), 192 (15), 191 (6), 190 (7), 180 (18), 179 (23), 169 (23), 165 (10), 164 (17), 152 (12), 150 (14), 149 (8), 141 (9), 140 (60), 136 (11), 125 (6), 120 (5), 110 (8), 108 (15), 81 (9), 80 (45), 57 (7); IR (CHCl<sub>3</sub>) 3385 (br), 15 2918, 2849, 1707, 1625, 1605, 1516, 1457, 1436, 1405, 1311, 1282, 1245, 1217, 1172, 1116, 1046, 1001, 968, 933, 874, 855, 666 cm<sup>-1</sup>.

(11aS)-7,8-Dimethoxy-2-(methoxycarbonylmethyl)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (42, UP2065, AN-SJG)

A catalytic amount of tetrakis(triphenylphosphine)palladium (44.0 mg, 38.0 μmol) was added to a stirred solution of the Alloc-protected carbinolamine 41 (0.66 g, 1.53 mmol), triphenylphosphine (20.0 mg, 77.0 μmol) and pyrrolidine (114 mg, 1.60 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (100 mL). After 2 h stirring at room temperature under a nitrogen atmosphere, TLC (99% CHCl<sub>3</sub>/MeOH) revealed the complete consumption of starting material. The

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solvent was evaporated in vacuo and the crude residue was purified by flash chromatography (98% CHCl3/MeOH) to afford the PBD (42, AN-SJG, UP2065) as an orange glass which was repeatedly evaporated in vacuo with CHCl3 in order to provide the N10-C11 imine form (481 mg, 95%):  $[\alpha]^{22}_{D} = +401.84$  ° (c = 1.00, CHCl<sub>3</sub>); <sup>1</sup>H 5 NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.87-7.85 (m, 1H), 7.49 (s, 1H), 6.93 (s, 1H), 6.81 (s, 1H), 4.34-4.27 (m, 1H), 3.95 (s, 3H), 3.93 (s, 3H), 3.74 (s, 3H), 3.34 (d, 1H, J = 16.85 Hz), 3.24 (s, 2H), 3.19-3.10(m, 1H); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  170.6, 162.7, 161.4, 151.8, 147.7, 140.4, 126.5, 119.0, 117.4, 111.5, 109.8, 56.2, 56.1, 10 53.8, 52.1, 37.4, 33.6; MS (EI), m/z (relative intensity) 332  $(M^{+} + 2, 5)$ , 331  $(M^{+} + 1, 9)$ , 330  $(M^{+}, 41)$ , 329 (28), 328 (100), 313 (18), 272 (8), 271 (24), 270 (14), 269 (27), 262 (7), 257 (12), 255 (5), 242 (6), 225 (7), 197 (4), 192 (16), 191 (16), 183 (6), 164 (14), 136 (11), 135 (9), 106 (9), 80 (17), 53 (5); 15 IR (CHCl<sub>3</sub>) 3329 (br), 3112, 2952, 2842, 1737, 1626, 1602, 1512, 1453, 1436, 1381, 1356, 1246, 1213, 1173, 1096, 1069, 1008, 875, 840, 786, 666, 620, 574, 537 cm<sup>-1</sup>; exact mass calcd for  $C_{17}H_{18}N_2O_5$ m/e 330.1216, obsd m/e 330.1237.

#### Example 1(f): Synthesis of KEC-570 (115, UP-2053) (see Figure 5)

#### 1', 3'-Bis(4-carboxy-2-methoxyphenoxy)propane (43)

A solution of diiodopropane (8.79 g, 29.7 mmol) in THF (50 mL), was added dropwise over a period of 4 h to a vigorously stirred solution of vanillic acid (10 g, 59.5 mmol) in THF (100 mL) and 5 aqueous NaOH (225 mL, 0.5 M) at 65°C in the absence of light (foil-wrapped flask). After heating at reflux for 48 h in the dark, the suspension was cooled, washed with hexane (3 x 100 mL) and the THF removed by evaporation in vacuo. The aqueous residue was acidified to pH 1 with conc. HCl and the resultant 10 precipitate collected by filtration, dried and recrystallised from glacial acetic acid to afford the corresponding biscarboxylic acid (118) as a white crystalline solid (9.4g, 84%). mp 238-240°C;  ${}^{1}H$ -NMR (DMSO- $d_6$ ):  $\delta$  2.23 (t, 2H, J = 6.0 Hz, H13), 3.80 (s, 6H,  $CH_3O$ ), 4.20 (t, 4H, J = 6.0 Hz, H12), 7.09 (d, 2H, J15 = 8.4 Hz, H10, 7.45 (d, 2H, J = 1.8 Hz, H6) 7.54 (dd, 2H, J =8.4 Hz, 1.8 Hz, H9), 12.76 (bs, 2H,  $CO_2H$ );  $^{13}C-NMR$  (DMSO- $d_6$ )  $\delta$ 28.4 (C13), 55.4 (CH<sub>3</sub>O), 64.8 (C12), 111.9 (C9), 112.0 (C6), 122.9 (C10), 123.0 (Q), 148.3 (Q), 151.6 (Q), 167.0 (C=O). IR (KBr):  $\nu = 3600-2000$ , 1680 (C=O), 1600 (C=C), 1515, 1465, 1430, 20 1345, 1310, 1270, 1225 (C-O-C), 1180, 1140, 1115, 1030, 990, 970, 950, 925, 875, 850, 825, 765, 725, 645 cm<sup>-1</sup>. MS (EI): m/z(relative intensity) 376 ( $M^+$ , 28), 360 (3), 249 (2), 209 (45), 165 (29), 153 (16), 151 (19), 137 (19), 121 (7), 78 (15), 44 (100); HRMS: Calcd for  $C_{19}H_{20}O_8 = 376.1158$  found 376.1168. 25

1',3'-Bis(4-carboxy-2-methoxy-5-nitrophenoxy)propane (44)

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The diacid 43 (2.0 g, 5.30 mmol) was added portionwise to conc.  $HNO_3$  (40 mL) at -10°C and stirred to room temperature over 12 h. The reaction mixture was poured on to ice (400 mL) and the resulting precipitate collected by filtration, washed with ether  $(3 \times 50 \text{ mL})$  and dried to afford the nitro acid (121) as a yellow solid (1.73 g, 70%). m.p. 243-246°C.  $^{1}$ H-NMR (DMSO- $d_6$ ):  $\delta$  2.25 (t, 2H, J = 5.9 Hz, H13), 3.90 (s, 6H, CH<sub>3</sub>O), 4.27 (t, 4H, J =5.9 Hz, H12), 7.29 (s, 2H, H6), 7.62 (s, 2H, H9), 13.6 (bs, 2H,  $CO_2H$ ). <sup>13</sup>C-NMR (DMSO- $d_6$ )  $\delta$  28.0 (C13), 56.3 (CH<sub>3</sub>O), 65.7 (C12), 108.0 (C9), 111.2 (C6), 121.1 (C5), 141.3 (Q), 149.1 (C8), 151.7 (Q), 165.9 (C=O). IR (KBr):  $\nu = 3620-2280$ , 1700 (C=O), 1595 (C=C), 1570, 1515  $(NO_2)$ , 1460, 1415, 1350  $(NO_2)$ , 1270, 1210, 1180, 1135, 1045, 930, 880, 810, 750, 730, 645 cm<sup>-1</sup>. MS (EI): m/z (relative intensity) 467 (MH<sup>+</sup>, 1), 450 (1), 436 (3), 423 (8), 378 (4), 268 (1), 255 (4), 236 (4), 210 (7), 194 (2), 182 (7), 164 (14), 153 (2), 123 (3), 91 (6), 77 (3), 55 (5), 44 (100). HRMS (EI) m/z calcd for  $C_{19}H_{18}N_2O_{12} = 466.0860$  found 466.0871.

20 (2s,4R)-N-(Benzoxycarbonyl)-2-carboxy-4-hydroxypyrrolidine (45)

A solution of benzyl chloroformate (12.5 mL, 87.7 mL) in toluene

(40 mL) was added to a solution of trans-4-hydroxy-L-proline 11

(10 g, 76.3 mmol) and NaHCO<sub>3</sub> (16 g, 190 mmol) in H<sub>2</sub>O (165 mL)

over a period of 15 min. After stirring at room temperature for

12 h the two phases were allowed to separate. The aqueous phase was washed with diethyl ether (4 x 50 mL), cooled in an ice bath,

and then acidified to pH 2 with conc. HCl. The resultant product was extracted with ethyl acetate (5 x 50 mL) and the combined organic extracts were dried (MgSO<sub>4</sub>) and the excess solvent evaporated in vacuo to afford a colourless viscous oil (20.30 g, 100%).  $[\alpha]^{27}_D = -565^\circ$  (c 0.1, MeOH). H NMR (CDCl<sub>3</sub>):  $\delta$  2.07-2.31 (m, 3H, H1), 3.52-3.59 (m, 2H, H3), 4.43-4.53 (m, 2H, H2, H11a), 5.8 and 5.11 (s, 2H, minor and major rotamers of H6, 1:2), 6.0 (bs, 2H, OH), 7.26-7.29 and 7.32-7.34 (m, 5H, minor and major rotamers of H arom, 1:2). IR (thin film):  $\nu$  = 3414 (OH), 2940 (OH), 1682 (C=O), 1495, 1429, 1359 (CO<sub>2</sub><sup>-</sup>), 1314, 1269, 1205, 1180, 1174, 1127, 1082, 1051, 993, 914, 866, 826, 769, 741, 697 cm<sup>-1</sup>. MS (EI): m/e (relative intensity): 266 (M<sup>+</sup>, 1), 265 (6), 220 (5), 176 (15), 130 (34), 108 (2). 91 (100), 86 (4), 68 (11). HRMS calcd. for C<sub>13</sub>H<sub>15</sub>NO<sub>5</sub> = 265.0950 found 265.0976

### 15 (2S,4R)-N-(Benzoxycarbonyl)-2-methyoxycarbonyl-4-hydroxyproline (46)

A solution of (2S,4R)-N-(Benzoxycarbony1)-2-carboxy-4- hydroxypyrrolidine (45) (20.30~g, 76.3~mmol) in dry methanol (300~mL) was heated at reflux for 18 h in the presence of a catalytic amount of conc.  $H_2SO_4$  (2.20~mL, 7.63~mmol). The reaction mixture was allowed to cool to room temperature and neutralised with  $Et_3N$  (3.0~mL, 76.3~mmol). The reaction mixture was concentrated in vacuo and the residue redissolved in ethyl acetate (200~mL), washed with brine (1~x~50~mL), dried  $(MgSO_4)$  and excess solvent removed under reduced pressure to afford a colourless gum (21.17~g, 99%).  $[\alpha]^{20}_{D} = -59.4^{\circ}$   $(c~0.014, CHCl_3)$ .  $^{1}H$  NMR  $(CDCl_3)$ :  $\delta~2.04-2.08$  and 2.24-2.35 (m, 1H, rotamers of H1,

1:1), 2.64 (bs, 1H, OH), 3.54 and 3.74 (s, 3H, rotamers of OMe, 1:1), 3.66-3.69 (m, 2H, H3), 4.47-4.50 (m, 2H, H2, H11a), 5.07-5.13 (m, 2H, H6), 7.26-7.35 (m, 5H, H arom). <sup>13</sup>C NMR (CDCl<sub>3</sub>): rotamer ratio 1:1,  $\delta$  37.8 and 38.5 rotamers of (C1), 51.8 and 52.0 rotamers of (OMe), 54.1 and 54.7 rotamers of (C3), 57.4 and 5 57.7 rotamers of (C2), 66.9 and 67.0 rotamers of (C6), 68.6 and 69.3 rotamers of (C11a), 127.0, 127.3, 127.4, 127.7, 127.8, 128.0 and 128.1 rotamers of (C arom). IR (thin film): v = 3435 (OH), 3033, 2953 (OH), 1750 ( ester), 1680 (C=O), 1586, 1542, 1498, 1422, 1357 (CO<sub>2</sub>H), 1170, 1124, 1084, 1052 (C-O), 1004, 963, 916, 10 823, 770, 750, 699, 673 cm<sup>-1</sup>. MS (FAB) m/z (relative intensity): 280 (M<sup>+</sup>·, 24), 236 (20), 234 (4), 216 (8), 214 (4), 213 (2), 206 (2), 204 (7), 203 (4), 202 (10), 201 (2), 181 (5), 144 (16), 102 (23), 91 (100). HRMS calcd. for  $C_{14}H_{17}NO_5 = 279.1107$  found . 15 279.1192

(2S,4R)-N-(Benzoxycarbony1)-2-hydroxymethy1-4-hydroxyproline (47)
Lithium borohydride (1.57 g, 73 mmol) was added portionwise to a
solution of (2S,4R)-N-(benzoxycarbony1)-2-methyoxycarbony1-4hydroxyproline (46) (20.17 g, 73 mmol) in THF (350 mL) at 0°C.

20 The reaction mixture was allowed to warm to room temperature and
stir overnight. The resulting suspension was cooled to 0°C and
quenched with water (2-3 mL) until effervescence ceased, at which
point 2 M HCl (15 mL) was added to dissolve the precipitate. The
product was extracted with ethyl acetate (3 x 150 mL) and the

25 combined organic phases washed with brine (1 x 100 mL) and then
dried (MgSO<sub>4</sub>). Concentration in vacuo afforded a white gum
(18.25 g, 100%). [α]<sup>22.3</sup><sub>D</sub> = -404° (C = 0.043, CHCl<sub>3</sub>). <sup>1</sup>H NMR

(CDCl<sub>3</sub>): δ 1.24-1.26 (m, 1H, H1), 1.73-2.08 (m, 1H, H1), 3.40-4.30 (m, 6H, H2, H3, H11, H11a), 5.06 (bs, 1H, OH), 5.09 (s, 2H, H6) 7.25-7.31 (m, 5H, H arom). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 36.7 (C1), 55.2 (C3), 58.7 (C2), 65.0 (C11), 67.0 (C6), 68.7 (C11a), 127.0, 127.5 (C arom), 127.8 (C arom), 128.2 (C arom). IR (thin film): ν = 3390 (OH), 3065, 3033, 2953 (OH), 1681 (C=O carbamate), 1586, 1538, 1498, 1454, 1192, 1122, 978, 914, 862, 770, 698, 673 cm<sup>-1</sup>. MS (FAB) m/z (relative intensity): 252 (M<sup>+</sup>, 58), 208 (33), 176 (5), 144 (6), 118 (8), 116 (7), 92 (13), 91 (100). HRMS calcd. for C<sub>13</sub>H<sub>17</sub>NO<sub>4</sub> = 251.1158 found 251.1230.

### (2S,4R)-N-Benzoxycarbonyl-2-t-butyldimethylsilyloxymethyl-4-hydroxypyrrolidine (48)

t-butyldimethylsilyl chloride (5.78 g, 38.3 mmol) and 1,8diazabicyclo[5,4,0]undec-7-ene (1.44 mL, 9.6 mmol) were added to 15 a solution of alcohol (47) (12.51 g, 49.8 mmol) and triethylamine (7.0 mL, 49.8 mmol) in dry DCM (200 mL) which had been allowed to stir for 15 min at room temperature. The resulting mixture was allowed to stir at room temperature for 18 h and then diluted with ethyl acetate (300 mL). The organic phase was washed with 20 aqueous saturated ammonium chloride (2 x 100 mL) and brine (1 x 100 mL) dried (MgSO<sub>4</sub>) and the solvent removed under reduced pressure to yield a colourless viscous oil (9.84 g, 70%).  $[\alpha]^{22.3}_{D} = -263^{\circ}$  (c 0.70, CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>) :  $\delta$  -0.05 and -0.06(s, 6H, rotamers of H1', H2', 1:1), 0.83 and 0.85 (s, 9H, rotamers of H3', H5', H6', 1:1), 1.95-2.22 (m, 2H, H1,), 2.78 25 (bs, 1H, OH), 3.44-3.68 (m, 3H, H3, H11), 3.99-4.10 (m, 1H, H2), 4.43-4.46 (m, 1H, H11a), 5.11-5.16 (m, 2H, H6) 7.34-7.35 (m, 5H,

H arom) <sup>13</sup>C NMR (CDCl<sub>3</sub>): rotamer ratio of 1:1,  $\delta$  -5.50 (C3', C5', C6'), 18.15 (C4'), 25.83 (C1', C2'), 36.55 and 37.27 rotamers of (C1), 55.2 and 55.7 rotamers of (C3), 57.3 and 57.8 rotamers of (C2), 62.8 and 63.9 rotamers of (C11), 66.6 and 67.0 rotamers of (C6), 69.7 and 70.3 rotamers of (C11a), 127.8 (C arom), 127.9 (C arom), 128.0 (C arom), 128.4 (C arom), 128.5 (C arom), 136.5 and 136.8 rotamers of (C7), 154.9 and 155.2 rotamers of (C5). IR (thin film):  $\nu$  = 3415 (OH), 3066, 3034, 2953 (OH), 2930, 2884, 2857, 1703 (C=O carbamate), 1587, 1498, 1424, 1360 (C-CH<sub>3</sub>), 1288 (CH<sub>3</sub>Si), 1255 (t-Bu), 1220, 1195 (t-Bu), 1118 (Si-O), 1057, 1003, 917, 836, 774, 751, 698, 670 cm<sup>-1</sup>. MS (EI/CI) m/e (relative intensity): 366 (M<sup>+</sup>·, 100), 308 (14), 258 (2), 91 (2).

#### (2S,4R)-2-t-butyldimethylsilyloxymethyl-4-hydroxypyrrolidine (2)

A slurry of 10% Pd/C (190 mg) in ethyl acetate (20 mL) was added 15 to a solution of TBDMS ether (48) (1.90 g, 5.19 mmol) in ethanol (100 mL). The reaction mixture was hydrogenated (Parr apparatus) for 16 h. The catalyst was removed by vacuum filtration through Celite and excess solvent was evaporated under reduced pressure to give a yellow oil in quantitative yield (1.20 g, 100%). 20  $[\alpha]^{22.2}_{D} = +35.6^{\circ} (c \ 0.042, CHCl_{3}).$  <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta - (0.07-0.08)$ (m, 6H, H1', H2'), 0.82 (s, 9H, H3', H4', H5'), 1.68-1.73 (m, 2H,H1,), 2.99-3.11 (m, 2H, H11), 3.47-3.50 (m, 3H, H11a, H3), 4.09 (bs, 1H, NH or OH), 4.32 (bs, 1H, NH or OH).  $^{13}$ C NMR (CDCl<sub>3</sub>):  $\delta$ -5.4 (C3', C5', C6'), 18.1 (C4'), 25.8 (C1', C2'), 37.4 (C1), 25  $54.6 \text{ (C11)}, 58.1 \text{ (C2)}, 64.6 \text{ (C3)}, 72.2 \text{ (C11a)}. IR (thin film): <math>\vee$ = 3330 (OH), 2928, 2857, 1557, 1421, 1331 (C-CH<sub>3</sub>), 1249 (CH<sub>3</sub>-Si),

1204 (t-Bu), 1191 (t-Bu), 1100 (Si-O),1073, 993, 713 cm<sup>-1</sup>. MS (CI) m/e (relative intensity): 232 (M<sup>+</sup>, 100), 230 (13), 174 (5), 133 (6), 86 (6).

1,1'-[[(Propane-1,3-diyl)dioxy]bis[2-nitro-5-methoxy-1,4-phenylene)carbonyl]]-bis[(2S,4R)-2-t-butyldimethylsilyloxymethyl-4-hydroxypyrrolidine (49)

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A catalytic amount of DMF (2 drops) was added to a stirred suspension of bis-nitroacid (44) (2.00 g, 4.28 mmol) and oxalyl chloride (0.94 mL, 10.70 mmol) in dry THF (20 mL), and the reaction mixture was allowed to stir for 4 h. After evaporation of excess THF in vacuo, the resultant yellow residue was dissolved in dry THF (20 mL) and added dropwise over a period of 25 min to a vigorously stirred suspension of amine (2) (2.47 g, 10.70 mmol),  $Et_3N$  (2.50 mL, 17.9 mmol) and ice/water (0.6 mL) cooled in an ice bath. The mixture was then allowed to warm to room temperature for a further 1.5 h. After removal of the THF by evaporation in vacuo, the residue was diluted with water (100 mL) and extracted with ethyl acetate (3 x 100 mL). The combined organic phase was washed with water (3 x 25 mL) and brine (3 x 25 mL), dried (MgSO $_4$ ), and the solvent removed in vacuo to afford a yellow oil which was purified by flash chromatography (3% MeOH/CHCl<sub>3</sub>) to afford the bis-amide (49) as a yellow solid (2.05g, 54%).  $[\alpha]^{23.8} p = -993^{\circ}$  (c 0.033, CHCl<sub>3</sub>). NMR (CDCl<sub>3</sub>):  $\delta$  -0.05 (s, 12H, H1', H2'), 0.80 (s, 18H, H3', H5', H6'), 1.96-1.99 (m, 2H, H1), 2.14-2.16(m, 2H, H1), 2.19-2.24 (m, 2H, H13), 2.85-2.89 (m, 2H, H2) 3.16-3.19 (m, 4H, H11), 3.63- $3.66 \, (m, 4H, H3), 3.81 \, (s, 6H, OMe), 3.99-4.10 \, (m, 2H, H3), 4.23$  (t, 4H, J = 5.3 Hz, H12), 4.38 (bs, 2H, OH); 5.20-5.25 (m, 2H, H11a), 6.65 (s, 2H, H6), 7.55 (s, 2H, H9). <sup>13</sup>C-NMR (CDCl<sub>3</sub>): δ - 5.35 (C1', C2'), 18.2 (C4'), 25.8 (C3', C5', C6'), 28.9 (C13), 36.1 (C1), 54.9 (CH<sub>3</sub>O), 56.6 (C4), 57.3 (C12), 65.0 (C3), 70.0 (C2), 108.0 (C6), 109.4 (C9), 128.2 (Q), 137.2 (Q), 148.1 (Q), 148.5 (Q), 154.5 (Q), 166.5 (Q). IR (thin film): ν = 3392 (OH), 2950, 2856, 1623 (C=O), 1577 (C arom), 1524 (NO<sub>2</sub>), 1459, 1432, 1381, 1338 (C-CH<sub>3</sub>), 1278 (CH<sub>3</sub>-Si), 1219 (t-Bu), 1184 (t-Bu), 1075 1053, 1004, 938, 914, 837, 778, 724, 668, 649, cm<sup>-1</sup>. MS (FAB) m/z (relative intensity): 894 (M<sup>+</sup>·, 8), 893 (19), 878 (6), 835 (2), 779 (9), 761 (6), 517 (3), 459 (5), 258 (7), 100 (3), 86 (4), 75 (29), 73 (100), 59 (17), 58 (6).

1,1'-[[(Propane-1,3-diyl)dioxy]bis[2-amino-5-methoxy-1,4-phenylene)carbonyl]]-bis[(2S,4R)-2-t-butyldimethylsilyloxymethyl-4-hydroxypyrrolidine (50)

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A slurry of 10% Pd/C (155 mg) in ethyl acetate (20 mL) was added to a solution of the bis-amide (49) (1.55 g, 1.73 mmol) in ethanol (100 mL). The reaction mixture was hydrogenated (Parr apparatus) for 16 h. The reaction mixture was filtered through Celite and the solvent was removed under reduced pressure to give a yellow oil (50) in quantitative yield (1.44 g, 100%). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 0.00 (s, 12H, H1', H2'), 0.88 (s, 18H, H3', H5', H6'), 2.00-2.25 (m, 6H, H1, H13), 3.50-3.72 (m, 12H, H2, H3, H11, H11a), 3.74 (s, 6H, OMe), 4.16-4.20 (m, 4H, H3), 4.30-4.35 (m, 4H, H12), 4.49 (bs, 2H, OH); 6.23 (s, 2H, H9), 6.64 (s, 2H, H6) <sup>13</sup>C-NMR (CDCl<sub>3</sub>): δ -5.5 (C1', C2'), 18.1 (C4'), 25.8 (C3', C5', C6'), 29.6 (C13), 35.2 (C1), 56.7 (CH<sub>3</sub>O), 62.2 (C4), 64.1 (C3),

70.0 (**c2**), 102.2 (**c9**), 112.6 (**c6**), 140.4 (**Q**), 141.1 (**Q**), 150.6 (**Q**), 170.1 (**Q**); IR (neat): v = 3359 (OH), 2929, 2856, 1621 (C=O), 1591 (C arom), 1469, 1433, 1406, 1358, 1346 (C-CH<sub>3</sub>), 1261 (CH<sub>3</sub>-Si), 1232 (*t*-Bu), 1175 (*t*-Bu), 1117, 1056, 1006, 866, 835, 776 cm<sup>-1</sup>. MS (FAB) m/z (relative intensity) : 834 (M<sup>+</sup>·, 13), 833 (18), 773 (9), 602 (13), 399 (7), 371 (34), 232 (9), 206 (22), 192 (14), 176 (13), 166 (44), 150 (8), 100 (10), 73 (100).

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1,1'-[[(Propane-1,3-diyl)dioxy]bis[2-amino-N-allyloxycarbonyl-5-methoxy-1,4-phenyl-ene)-carbonyl]]-bis[(2S,4R)-2-t-

butyldimethylsilyloxymethyl-4-hydroxy-pyrrolidine (51)

A solution of the bis-amide (50) (2.76 g, 3.31 mmol) and pyridine (1.10 mL, 13.60 mmol) in dried DCM (100 mL) was cooled to 0°C. Allyl chloroformate (0.80 mL, 7.53 mmol) in DCM (50 mL) was added dropwise and the resulting mixture allowed to warm to room temperature and stirred for 16h. The reaction mixture was 15 diluted with DCM (200 mL) and washed with 1 M CuSO<sub>4</sub> (3  $\times$  50 mL), water (1 x 50 mL) and brine (1 x 50 mL) before drying (MgSO<sub>4</sub>). Evaporation of the solvent under reduced pressure followed by flash column chromatography (2.5% MeOH/DCM) afforded (51) as a yellow solid (3.24 g, 97%).  $[\alpha]^{20.1}_{D} = -571^{\circ}$  (c 0.007, CHCl<sub>3</sub>). 20  $^{1}$ H NMR (CDCl<sub>3</sub>): δ 0.00 (s, 12H, **H1'**, **H2'**), 0.89 (s, 18H, **H3'**, H5', H6'), 2.03-2.36 (m, 6H, H1, H13), 3.51-3.58 (m, 6H, H2, H3), 3.77 (s, 6H, OMe), 4.20-4.26 (m, 8H, H11, H12), 4.28-4.30 (m, 2H, H11a), 4.56-4.60 (m, 6H, H8', OH), 5.25 (dd,  $J_{1,2}=1.5$  Hz,  $J_{1,3}=$ 15.0 Hz, 4H, H10'), 5.90-5.95 (m, 2H, H9'), 6.73 (s, 2H, H6), 25 7.63 (s, 2H, **H9**), 8.80 (s, 2H, **NH**).  $^{13}$ C NMR (CDCl<sub>3</sub>):  $\delta$  -5.42 (C1', C2'), 25.8 (C3', C5', C6'), 29.2 (C13), 35.4 (C1), 56.3

(CH<sub>3</sub>O), 57.1 (Cl1a), 59.8 (Cl1), 62.2 (C3), 65.1 (Cl2), 65.7 (C8'), 70.5 (C2), 106.3 (C9), 111.5 (C6), 116.5 (Q), 118.1 (Cl0'), 131.7 (Q), 132.5 (C9'), 144.3 (Q), 150.3 (Q), 153.8 (Q), 169.5 (Q). IR (neat): v = 3351 (OH), 2931, 2857, 1762 (Alloc C=O), 1722, 1603 (C=O), 1521 (C arom), 1463, 1404, 1264 (CH<sub>3</sub>-Si), 1222 (t-Bu), 1106 (t-Bu), 1053, 1015, 936, 872, 837, 775, 629, cm<sup>-1</sup>.

1,1'-[[(Propane-1,3-diyl)dioxy]bis[2-amino-N-allyloxycarbonyl-5-methoxy-1,4-phenylene)-carbonyl]]-bis[(2S)-2-t-

10 butyldimethylsilyloxymethyl-4-oxo-pyrrolidine (52)

A solution of dimethyl sulphoxide (2.10 mL, 28.5 mmol) in dry DCM (20 mL) was added dropwise over a 15 min period to a stirred, cooled (-45°C) solution of oxalyl chloride (1.27 mL, 14.60 mmol) in DCM (30 mL). After 35 min, a solution of alcohol (51) (2.54g, 2.53 mmol) in DCM (20 mL) was added dropwise over a period of 15 15 min to the reaction mixture at -45°C. After 45 min a solution of triethylamine (5.75 mL, 40.3 mmol) in DCM (20 mL) was added over a period of 15 min and the reaction mixture stirred at -45°C for 30 min before warming to room temperature over 45 min. mixture was then washed with 1 M  $CuSO_4$  (3 x 50 mL), water (2 x 20 50 mL) and brine (1 x 50 mL) before drying (MgSO<sub>4</sub>) and concentrating in vacuo to give (52) as a yellow solid (2.46q, 97%). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  0.00 (s, 12H, H1', H2'), 0.86 (s, 18H, H3', H5', H6'), 2.50 -2.63 (m, 6H, H1, H13), 3.63-3.70 (m, 4H, H3), 3.80 (s, 6H, OMe), 3.93-3.97 (m, 6H, H11, H11a), 4.29-4.32 25 (m, 4H, H12), 4.62 (d, 4H, J = 5.7 Hz, H8'), 5.27-5.32 (m, 4H, H12)H10'), 5.98-6.03 (m, 2H, H9'), 6.74 (s, 2H, H6), 7.74 (s, 2H,

H9), 8.80 (s, 2H, NH).  $^{13}$ C NMR (CDCl<sub>3</sub>):  $\delta$  -5.76 (C1', C2'), 18.0 (C4'), 25.7 (C3', C5', C6'), 28.8 (C13), 39.6 (C1), 55.0 (C3), 56.4 (CH<sub>3</sub>O), 65.3 (C12), 65.8 (C8'), 105.9 (C9), 110.7 (C6), 118.2 (C10'), 132.4 (C9'), 150.7 (Q), 153.5 (Q), 169.1 (Q), 210.0 (C2). IR (neat):  $\nu$  = 3308 (OH), 2931, 2856, 1765 (Alloc C=O), 1730, 1624 (C=O), 1602 (C=O), 1522 (C arom), 1468, 1407, 1332, 1259 (CH<sub>3</sub>-Si), 1204 (t-Bu), 1105 (t-Bu), 1053, 1010, 937, 870, 837, 808, 778, 674, 657 cm<sup>-1</sup>.

1,1'-[[(Propane-1,3-diyl)dioxy]bis[2-amino-N-allyloxycarbonyl-510 methoxy-1,4-phenylene)-carbonyl]]-bis[(2S)-2-tbutyldimethylsilyloxymethyl-4-methoxycarbonyl methyl-2,3dihydropyrrole (53)

A solution of diethylmethylphosphonoacetate (0.80 mL, 4.21 mmol) in THF (50 mL) was added to a suspension of NaH (343 mg, 4.21 mmol, 60% dispersion in mineral oil, washed with petroleum ether) in dry THF (50 mL) at 0°C under a nitrogen atmosphere. After stirring at room temperature for 1 h, a solution of the dimer ketone (52) (2.04 g, 2.00 mmol) in THF (50 mL) was added dropwise at 0°C. The reaction mixture was allowed to warm to room temperature over 18 h. Excess THF was removed under reduced pressure and the residue cooled in an ice bath before adding NaHCO<sub>3</sub> (50 mL) followed by EtOAc (50 mL). The layers were separated and the aqueous layer washed with EtOAc (2 x 50 mL). The combined organic layers were washed with brine (1 x 50 mL), dried (MgSO<sub>4</sub>) and the solvent removed in vacuo to give a yellow oil. Flash column chromatography (2.5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) afforded the product (53) as a yellow solid (2.00 g, 88%). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ

-0.01 (s, 12H, H1', H2'), 0.83 (s, 18H, H3', H5', H6'), 2.35-2.40 (m, 2H, H13), 2.65-2.86 (m, 4H, H1), 3.03-3.09 (m, 4H, H14), 3.62(s, 3H, OMe), 3.75 (s, 6H, H16), 3.95-4.10 (m, 4H, H11), 4.24-4.35 (m, 4H, H12), 4.58-4.70 (m, 6H, H8', H11a), 5.25-5.33 (m, 4H, H10'), 5.93-5.97 (m, 2H, H9'), 6.33-6.40 (m, 2H, H3), 6.74 5 (s, 2H, H6), 7.80 (s, 2H, H9), 8.75 (s, 2H, NH). <sup>13</sup>C NMR  $(CDCl_3): \delta -5.52 (C1', C2'), 18.0 (C4'), 25.7 (C3', C5', C6'),$ 28.7 (C13), 33.8 (C14), 34.6 (C1), 51.9 (CH<sub>3</sub>O), 56.5 (C16), 62.2 (C11), 65.2 (C12), 65.6 (C8'), 105.4 (C9), 111.9 (C6), 117.9 (C10'), 128.2 (C3), 132.5 (C9'), 143.9 (Q), 150.7 (Q), 153.4 (Q), 10 165.7 (Q), 170.6 (Q). IR (neat): v = 3402 (OH), 2954, 2857, 1735 (ester), 1726 (Alloc C=O), 1642, 1600, 1526 (C arom), 1469, 1435, 1354, 1256 (CH<sub>3</sub>-Si), 1221, 1201 (t-Bu), 1112 (t-Bu), 1048, 1010, 934, 866, 836, 776  $\text{cm}^{-1}$ . MS (FAB) m/z (relative intensity): No parent ion, 496 (10), 482 (9), 455 (11), 441 (13), 232 (12), 206 15 (19), 204 (10), 200 (14), 192 (34), 188 (23), 172 (33), 165 (18), 152 (17), 150 (16), 149 (100), 147 (17), 140 (20), 131 (18), 103 (22), 91 (47), 89 (27), 87 (36), 80 (33), 75 (42), 73 (77), 61 (39), 57.(53).

1,1'-[[(Propane-1,3-diyl)dioxy]bis[2-amino-N-allyloxycarbonyl-5-methoxy-1,4-phenylene)-carbonyl]]-bis[(2s)-2-hydroxymethyl-4-methoxycarbonylmethyl-2,3-dihydropyrrole (54)

Hydrofluoric acid.pyridine complex (3.5 mL) was added to a solution of dimer ester (53) (740 mg, 0.67 mmol) in THF (10 mL)

under a nitrogen atmosphere at 0°C. The reaction was allowed to stir for 30 min at 0°C and then to warm to room temperature over 1 h. The reaction mixture was neutralised with NaHCO3 until

evolution of  ${\rm CO_2}$  ceased. The product was extracted with DCM (3  $\times$ 30 mL), washed with brine (1 x 20 mL) and then dried (MgSO<sub>4</sub>). Removal of solvent under reduced pressure gave the product as a yellow gum (530 mg, 90%).  $^{1}$ H NMR (CDCl<sub>3</sub>):  $\delta$  2.39 (m, 2H, H13), 2.95-2.99 (m, 4H, H1), 3.09-3.12 (m, 4H, H14), 3.68 (s, 3H, OMe), 5 3.74-3.78 (m, 4H, H11), 3.81 (s, 6H, H16), 4.28-4.34 (m, 4H, H12), 4.62 (d, J = 5.5 Hz, 4H, H8'), 4.73-4.75 (m, 2H, H11a), 5.31-5.38 (m, 4H, H10'), 5.96-6.02 (m, 2H, H9'), 6.39-6.50 (m, 2H, H3), 6.80 (s, 2H, H6), 7.72 (s, 2H, H9), 8.57 (s, 2H, NH). 10 <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  28.8 (**C13**), 33.5 (**C14**), 35.5 (**C1**), 52.1  $(CH_3O)$ , 56.6 (C16), 65.3 (C12), 66.0 (C8'), 105.6 (C9), 111.8 (C6), 118.1 (C10'), 128.1 (C3), 132.5 (C9'), 144.4 (Q), 151.0 (Q), 153.6 (Q), 167.3 (Q), 170.7 (Q). IR (neat): v = 3416 (OH), 2953, 1731 (ester), 1726 (Alloc C=O), 1606, 1525 (C arom), 1467, 15 1434, 1358, 1224, 1048, 938, 870, 768  $cm^{-1}$ . MS (FAB) m/z (relative intensity): 881 (M+, 0.2), 496 (12), 482 (15), 456 (14), 442 (13), 232 (23), 206 (35), 192 (63), 190 (21), 188 (17), 180 (19), 178 (25), 152 (39), 150 (23), 149 (100), 140 (50), 136 (21), 112 (23), 108 (23), 94 (29), 91 (32), 87 (24), 80 (70), 73 20 (28), 57 (30).

1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11aS)-7-methoxy-10-allyloxycarbonyl-(2S)-2-methoxycarbonylmethyl-2,3-dihydropyrrole-1,3,11a-trihydro-5H-pyrrolo[2,1-c][1,4]bezodiazepin-5-one (55)

A solution of dimethyl sulphoxide (0.27 mL, 3.82 mmol) in dried

DCM (10 mL) was added dropwise over a 15 min period to a stirred, cooled (-45°C) solution of oxalyl chloride (0.17 mL, 1.92 mmol) in DCM (10 mL). After 35 min, a solution of substrate (54) (600

mg, 0.68 mmol) in DCM (10 mL) was added dropwise over a period of 15 min to the reaction mixture at -45 °C. After 45 min a solution of triethylamine (0.78 mL, 5.42 mmol) in DCM (10 mL) was added over a period of 15 min and the reaction mixture stirred at -45°C 5 for 30 min before being allowed to warm to room temperature over 45 min. The mixture was then diluted with water (10 mL) and the layers separated. The organic layer was washed with 1 M HCl (3  $\times$ 50 mL), and brine (1 x 50 mL) before drying (MgSO<sub>4</sub>) and concentrating in vacuo. Flash column chromatography (1.5% 10 MeOH/CH<sub>2</sub>Cl<sub>2</sub>) afforded a yellow glass (457 mg, 78%).  $[\alpha]^{20.3}$  =  $+69^{\circ}$  (c 0.484, CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  2.35-2.63 (m, 2H, H13), 2.75-3.10 (m, 4H, H1), 3.14-3.19 (m, 4H, H14), 3.71 (s, 3H, OMe), 3.88 (s, 6H, H16), 4.21-4.40 (m, 4H, H12), 4.45-4.50 (m, 2H, H11a), 4.60-4.62 (m, 4H, H8'), 5.26-5.30 (m, 4H, H10'), 5.77 (d, J = 8.61 Hz, 4H, H11) 5.90-5.96 (m, 2H, H9'), 6.75-6.80 (m, 2H,15 **H3**), 6.89 (s, 2H, **H9**), 7.22 (s, 2H, **H6**).  $^{13}$ C NMR (CDCl<sub>3</sub>):  $\delta$  28.8 (C13), 33.5 (C14), 35.5 (C1), 52.1 (CH<sub>3</sub>O), 56.6 (C16), 65.3 (C12), 66.0 (C8'), 105.6 (C9), 111.8 (C6), 118.1 (C10'), 128.1 (C3), 132.5 (C9'), 144.4 (Q), 151.0 (Q), 153.6 (Q), 167.3 (Q), 20 170.7 (Q). IR (neat): v = 3583, 3412 (OH), 1730 (ester), 1713 (Alloc C=O), 1644, 1421, 1362, 1273, 1223, 1092, 902, 757, 737, 702, 667 cm<sup>-1</sup>. MS (FAB) m/z (relative intensity):  $907 (M^+, 1)$ , 456 (6), 245 (7), 232 (16), 218 (13), 206 (23), 205 (10), 204 (14), 192 (42), 190 (17), 178 (22), 177 (10), 176 (16), 166 (17), 25 165 (10), 164 (16), 152 (23), 151 (12), 150 (18), 149 (100), 140 (16), 93 (18), 91 (22), 89 (13), 87 (26), 80 (58), 75 (19), 73 (28), 57 (25).

1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11aS)-7-methoxy-(2S)-2-methoxycarbonylmethyl-2,3-dihydropyrrole-1,3,11a-trihydro-5H-pyrrolo[2,1-c][1,4]bezodiazepin-5-one (56)

A catalytic amount of tetrakistriphenylphosphinepalladium(0) (16 mg, 0.014 mmol) was added to a solution of carbinolamine (55) 5 (219 mg, 0.25 mmol), triphenylphosphine (7 mg, 0.025 mmol) and pyrrolidine (0.05 mL, 0.80 mmol) in dry DCM (30 mL) at 0°C. reaction mixture was stirred for 2 h before being allowed to warm to room temperature over 1 h. The solvent was removed in vacuo and the residue was subjected to flash column chromatography (2% 10  $MeOH/CH_2Cl_{21}$  R<sub>f</sub> = 0.25 ) to yield a yellow glass (109 mg, 66%).  $[\alpha]^{19.5}_{D} = +500^{\circ} (c \ 0.043, \ CHCl_{3}).$  <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta \ 2.17-2.42$  (m, 2H, H13), 3.15-3.32 (m, 8H, H1, H14), 3.73 (s, 3H, OMe), 3.91 (s, 6H, H16), 4.26-4.30 (m, 6H, H12, H11a), 6.84 (s, 2H, H9), 6.92-7.06 (m, 2H, H3), 7.47 (s, 2H, H6), 7.83 (d, J = 4.0 Hz, 4H, 15 H11).  $^{13}$ C NMR (CDCl<sub>3</sub>):  $\delta$  28.7 (C13), 33.6 (C14), 37.4 (C1), 52.2  $(CH_3O)$ , 53.8 (C11), 56.2 (C16), 65.4 (C12), 110.9 (C9), 111.8 (C6), 126.5 (C3), 140.2 (Q), 148.0 (Q), 151.0 (Q), 161.4 (Q), 162.6 (C11a), 170.7 (Q). IR (neat): v = 3583, 3394, 2997, 2950, 1736 (ester), 1717 (Alloc C=O), 1628, 1596, 1511, 1483, 1451, 20 1431, 1382, 1273, 1245, 1197, 1152, 1068, 995, 963, 914, 842, 753  $cm^{-1}$ . FABMS m/z (relative intensity): 673 (M<sup>+</sup>·, 2), 279 (6), 277 (4), 201 (7), 185 (55), 181 (7), 110 (5), 93 (100), 91 (24), 75 (28), 73 (20), 61 (12), 57 (33).

### Example 2(a): Synthesis of the C7-Iodo-C2-methlene PBD Monomer BSD-SJG (64, UP-2023) (see Figure 6)

# (S) -N- (Allyloxycarbonyl) -2- (tert-butyldimethylsilyloxymethyl) -4- methylidenepyrrolidine (57)

Potassium tert-butoxide (41.0 mL of a 0.5 M solution in THF, 20.5 5 mmol) was added dropwise to a suspension of methyltriphenylphosphonium bromide (7.29 g, 20.4 mmol) in THF (20 mL) at 0°C (ice/acetone) under nitrogen. After stirring for 2 h at 0°C, a solution of the ketone 16 (example 1(b)) (3.20 g, 10.2 mmol) in THF (10 mL) was added dropwise and the mixture 10 allowed to warm to room temperature. After stirring for a further 30 min the reaction mixture was diluted with EtOAc (150 mL) and water (150 mL) and the organic layer separated, washed with brine, dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo to give a yellow oil in which crystals (TPO) formed upon standing in the freezer. Purification by flash chromatography (5% EtOAc/Petroleum Ether) isolated the pure olefin 57 as a colourless oil (2.76 g, 87%):  $[\alpha]^{21}_{D} = -22.2$  ° (c = 0.25, CHCl<sub>3</sub>);  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>) (Rotamers)  $\delta$  6.02-5.87 (m, 1H,  $NCO_2CH_2CH=CH_2$ ), 5.31 (ddd, 1H, J = 1.65, 3.11, 17.20 Hz, 20  $NCO_2CH_2CH=CH_2$ ), 5.21 (dd, 1H, J=1.46, 10.40 Hz,  $NCO_2CH_2CH=CH_2$ ), 4.99-4.61 (m, 2H, NCH<sub>2</sub>C=CH<sub>2</sub>), 4.60 (d, 2H, J = 4.94 Hz,  $NCO_2CH_2CH=CH_2$ ), 4.19-3.98 (m, 2H,  $NCHCH_2OTBDMS$ ), 3.93-3.87 (m, 1H, NCHCH2OTBDMS), 3.66-3.42 (m, 2H, NCH2C=CH2), 2.80-2.56 (m, 2H,  $NCH_2C=CH_2CH_2$ ), 0.87 (s, 9H,  $SiC(CH_3)_3$ ), 0.03-0.02 (m, 6H, 25

Si(CH<sub>3</sub>)<sub>2</sub>); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>) (Rotamers)  $\delta$  154.4 (NC=O), 145.1 and 144.1 (NCH<sub>2</sub>C=CH<sub>2</sub>), 133.1 (NCO<sub>2</sub>CH<sub>2</sub>CH=CH<sub>2</sub>), 117.5 and 117.2 (NCO<sub>2</sub>CH<sub>2</sub>CH=CH<sub>2</sub>), 107.5 and 106.9 (NCH<sub>2</sub>C=CH<sub>2</sub>), 65.8 and 65.6 (NCO<sub>2</sub>CH<sub>2</sub>CH=CH<sub>2</sub>), 63.7 and 63.1 (NCHCH<sub>2</sub>OTBDMS), 58.7 and 58.3 (NCHCH<sub>2</sub>OTBDMS), 51.1 (NCH<sub>2</sub>C=CH<sub>2</sub>), 34.9 and 34.2 (NCH<sub>2</sub>C=CH<sub>2</sub>CH<sub>2</sub>), 25.8 (SiC(CH<sub>3</sub>)<sub>3</sub>), 18.2 (SiC(CH<sub>3</sub>)<sub>3</sub>), -5.5 (Si(CH<sub>3</sub>)<sub>2</sub>); MS (CI), m/z (relative intensity) 312 (M<sup>+</sup> + 1, 82), 296 (9), 279 (5), 255 (20), 254 (M-OC<sub>3</sub>H<sub>5</sub> or M-<sup>t</sup>Bu, 100), 168 (8), 122 (14); IR (Neat) 3083 (C=CH<sub>2</sub>), 2954, 2847, 1709 (NC=O), 1533, 1467, 1404 (SiCH<sub>3</sub>), 1360, 1310, 1252 (SiCH<sub>3</sub>), 1207, 1174, 1103, 1076, 1006, 836, 776, 680 cm<sup>-1</sup>.

# (2S)-2-(tert-butyldimethylsilyloxymethyl)-4-methylidenepyrrolidine (50)

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A catalytic amount of  $PdCl_2(PPh_3)_2$  (92 mg, 0.131 mmol) was added to a solution of the allyl carbamate **57** (1.0 g, 3.22 mmol) and  $H_2O$  (0.34 mL, 18.9 mmol) in  $CH_2Cl_2$  (30 mL). After 5 min stirring at room temperature,  $Bu_3SnH$  (0.96 mL, 1.04 g, 3.57 mmol) was added rapidly in one portion. A slightly exothermic reaction with vigorous gas evolution immediately ensued. The mixture was stirred for 16 h at room temperature under nitrogen at which point TLC (50% EtOAc/Petroleum Ether) revealed the formation of amine. After diluting with  $CH_2Cl_2$  (30 mL), the organic solution was dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo* to give an orange oil which was purified by flash chromatography (50-100% EtOAc/Petroleum Ether) to afford the amine **58** as a slightly orange oil (0.56 g, 77%):  $[\alpha]^{21}_D = -3.9$ ° (c = 5.0,  $CHCl_3$ ); <sup>1</sup>H NMR (270 MHz,  $CDCl_3$ )  $\delta$  4.93 (t, 1H, J = 2.02 Hz,  $NCH_2C=CH_2$ ), 4.90 (t,

1H, J = 2.02 Hz,  $NCH_2C=CH_2$ ), 3.68-3.46 (m, 4H,  $NCHCH_2OTBDMS$  and  $NCH_2C=CH_2$ ), 3.30-3.21 (m, 1H,  $NCHCH_2OTBDMS$ ), 2.53-2.41 (m, 2H,  $NCH_2C=CH_2CH_2$  and NH), 2.26-2.17 (m, 1H,  $NCH_2C=CH_2CH_2$ ), 0.90 (s, 9H,  $SiC(CH_3)_3$ ), 0.06 (s, 6H,  $Si(CH_3)_2$ );  $^{13}C$  NMR (67.8 MHz,  $CDCl_3$ )

5  $\delta$  150.0 ( $NCH_2C=CH_2$ ), 104.7 ( $NCH_2C=CH_2$ ), 64.7 ( $NCHCH_2OTBDMS$ ), 60.5 ( $NCHCH_2OTBDMS$ ), 51.3 ( $NCH_2C=CH_2$ ), 34.9 ( $NCH_2C=CH_2CH_2$ ), 25.9 ( $SiC(CH_3)_3$ ), 18.3 ( $SiC(CH_3)_3$ ), -5.4 ( $Si(CH_3)_2$ ); MS (EI), m/z (relative intensity) 227 ( $M^+$ , 8), 212 (6), 170 ( $M^-$ tBu, 36), 96 (8), 82 ( $M^-$ CH\_2OTBDMS, 100), 75 (11); IR (Neat) 3550-3100 (br, NH), 3074 ( $C=CH_2$ ), 2929, 2857, 1664 (C=C), 1472, 1424, 1391, 1380, 1361, 1255, 1190, 1101, 1006, 939, 880, 838, 777, 723, 668 cm<sup>-1</sup>.

(2S)-N-[5-Iodo-2-(2,2,2-trichloroethyloxycarbonylamino)-benzoyl]-2-(tert-butyldimethylsilyloxymethyl)-4-methylidinepyrrolidine (60)

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A catalytic amount of DMF (3 drops) was added to a stirred solution of the Troc protected anthranilic acid 59 (0.46 g, 1.04 mmol) and oxalyl chloride (0.10 mL, 0.15 g, 1.15 mmol) in  $\mathrm{CH_2Cl_2}$  (30 mL). After 16 h at room temperature the resulting acid chloride solution was added dropwise over 30 min to a stirred mixture of the amine 58 (0.26 g, 1.15 mmol) and TEA (0.26 g, 0.36 mL, 2.58 mmol) in  $\mathrm{CH_2Cl_2}$  (15 mL) at  $-20^{\circ}\mathrm{C}$  ( $\mathrm{CCl_4/liq.N_2}$ ) under a nitrogen atmosphere. The reaction mixture was allowed to warm to room temperature and stirred for a further 45 min. At this point TLC analysis (50% EtOAc/Petroleum Ether) revealed complete reaction. The mixture was washed with saturated NaHCO<sub>3</sub> (30 mL), saturated NH<sub>4</sub>Cl (30 mL), H<sub>2</sub>O (25 mL), brine (30 mL), dried

(MgSO<sub>4</sub>), filtered and evaporated *in vacuo* to give the amide **60** as a dark oil (0.65 g, 96%):  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>) (Rotamers)  $\delta$  8.92 (br s, 1H), 8.05-7.88 (m, 1H), 7.74-7.64 (m, 1H), 7.56-7.46 (m, 1H), 5.08-4.95 (m, 2H), 4.84 (d, 1H, J=11.91 Hz), 4.75 (d, 1H, J=11.91 Hz), 4.74-4.65 (m, 1H), 4.21-3.68 (m, 4H), 2.96-2.65 (m, 2H), 0.95-0.87 (m, 9H), 0.1-0.03 (m, 6H).

# (2S) - N - (2-Amino-5-iodobenzoy1) - 2 - (hydroxymethy1) - 4-methylidenepyrrolidine (61)

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A solution of TBAF (1.24 mL of a 1M solution in THF, 1.24 mmol) was added to the silyl-ether 60 (0.64 g, 0.99 mmol) in THF 10 (15 mL) at 0°C (ice/acetone). The reaction mixture was allowed to warm to room temperature and after 45 min TLC (50% EtOAc/Pet-Ether 40 °- 60 °) revealed the complete disappearance of starting material. Saturated NH<sub>4</sub>Cl (75 mL) was added and the reaction mixture extracted with EtOAc (3 X 30 mL), washed with brine 15 (30 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo to give an orange oil. Purification by flash chromatography (50% EtOAc/Pet-Ether 40 °- 60 °) provided the pure amino-alcohol 61 as a viscous oil (0.18 g, 51%):  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.72-7.61 (m, 1H), 7.55-7.40 (m, 1H), 6.51-6.49 (m, 1H), 5.02-4.94 (m, 2H), 20 4.80-3.80 (m, 8H), 2.81-2.79 (m, 1H), 2.43-2.40 (m, 1H); MS (EI), m/z (relative intensity) 359 (M<sup>+</sup>· + 1, 5), 358 (M<sup>+</sup>·, 33), 328 (3), 327 (10), 254 (3), 247 (11), 246 (100), 218 (18), 164 (2), 127 (4), 120 (4), 119 (10), 113 (9), 112 (91), 94 (2), 91 (20), 90 (5), 82 (10), 67 (2), 64 (3), 63 (3), 52 (3). 25

(2S) -N-[5-Iodo-2-(2,2,2-trichloroethyloxycarbonylamino)-benzoyl]-2-(hydroxymethyl)-4-methylidinepyrrolidine (62).

A solution of the amine 61 (179 mg, 0.50 mmol) in  $CH_2Cl_2$  (15 mL) was cooled to 0°C (ice/acetone) and treated with pyridine (81 µL, 79 mg, 1.0 mmol). A solution of 2,2,2-5 trichloroethylchloroformate (76 µL, 117 mg, 0.55 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was then added dropwise to the stirred mixture. reaction mixture was allowed to warm to room temperature and stirred for a further 2 h, at which point TLC (EtOAc) revealed complete consumption of amine 61. The reaction mixture was 10 washed with saturated  $CuSO_4$  (25 mL),  $H_2O$  (25 mL), brine (25 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo. The crude residue was purified by flash chromatography (50% EtOAc/Petroleum Ether) to afford the pure troc-amino compound 62 as an oil (189 mq, 71%): <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  8.90 (br s, 1H), 7.75-7.66 15 (m, 3H), 5.02-4.92 (m, 3H), 4.87 (d, 1H), J = 12.09 Hz, 4.72 (d, 1H)1H, J = 12.09 Hz), 4.15-4.08 (m, 2H), 3.90-3.85 (m, 2H), 3.65-3.63 (m, 1H), 2.80-2.71 (m, 1H), 2.50 (d, 1H, J = 14.83 Hz); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>) δ 167.7, 151.9, 142.7, 139.6, 135.6, 134.8, 20 127.7, 123.4, 108.4, 95.1, 86.6, 74.3, 63.9, 59.0, 53.5, 33.7; MS (EI), m/z (relative intensity) 536 (5), 535 (3), 534 (15), 533  $(M^+, 3), 532 (15), 503 (2), 501 (2), 422 (4), 420 (5), 385 (8),$ 384 (8), 366 (3), 353 (11), 290 (9), 273 (8), 272 (76), 246 (6), 245 (18), 218 (4), 217 (5), 216 (8), 146 (4), 145 (10), 133 (4), 131 (4), 119 (6), 117 (7), 115 (11), 113 (17), 112 (39), 97 (4), 25 96 (3), 95 (12), 90 (5), 84 (5), 83 (7), 82 (100), 79 (7), 77 (21), 67 (2), 63 (4), 61 (3), 51 (6); exact mass calcd for  $C_{16}H_{16}N_2O_4Cl_3I$  m/e 531.9221, obsd m/e 531.9155.

(11S,11aS)-11-Hydroxy-7-iodo-2-methylidene-10-(2,2,2-trichloroethyloxycarbonylamino)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (63)

A solution of the alcohol 62 (189 mg, 0.35 mmol) in  $CH_2Cl_2/CH_3CN$ (12 mL, 3:1) was treated with 4  $\mathring{A}$  powdered molecular sieves (100 5 mg) and NMO (62 mg, 0.53 mmol). After 15 min stirring at room temperature, TPAP (6.2 mg, 17.7 µmol) was added and stirring continued for a further 1 h at which point TLC (50% EtOAc/Petroleum Ether) showed product formation along with some unoxidised starting material. The mixture was then treated with 10 a further quantity of NMO (62 mg, 0.53 mmol) and TPAP (6.2 mg, 17.7 µmol) and allowed to stir for a further 30 min after which time TLC revealed complete reaction. The mixture was evaporated in vacuo onto silica and subjected to flash chromatography (40% EtOAc/Petroleum Ether) to provide the protected carbinolamine 63 15 as a white glass (93 mg, 49%):  $^{1}\text{H}$  NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  8.09 (d, 1H, J = 2.01 Hz), 7.80 (dd, 1H, J = 8.43, 2.20 Hz), 7.10 (d, 1H, J = 8.43 Hz), 5.60 (d, 1H, J = 9.71 Hz), 5.20-5.04 (m, 3H), 4.79-4.50 (m, 1H), 4.32-4.08 (m, 3H), 3.63 (t, 1H, J = 8.79 Hz), 2.99-2.89 (m, 1H), 2.72 (d, 1H, J = 15.94 Hz); <sup>13</sup>C NMR (67.8 MHz, 20  $CDCl_3$ )  $\delta$  165.0, 154.1, 141.0, 140.2, 137.7, 134.5, 133.6, 132.0, 110.4, 94.7, 93.4, 85.7, 75.0, 59.4, 50.7, 35.0; MS (EI), m/z (relative intensity) 533 (6), 532 (22), 531 (M+·, 8), 530 (17), 529 (10), 449 (5), 383 (6), 354 (7), 353 (5), 338 (6), 325 (5), 290 (5), 274 (15), 273 (8), 272 (43), 254 (5), 245 (8), 218 (5), 25 216 (12), 147 (5), 146 (6), 145 (9), 133 (10), 131 (9), 128 (5), 127 (15), 119 (11), 117 (5), 97 (6), 95 (9), 92 (6), 91 (6), 90 (6), 83 (11), 82 (100), 81 (7), 80 (8), 75 (5), 63 (7), 53 (5);

exact mass calcd for  $C_{16}H_{14}N_2O_4ICl_3$  m/e 531.9037, obsd m/e 531.8988.

(11aS) -7-Iodo-2-methylidene-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (64, UP2023, BSD-SJG).

5 10% cadmium-lead couple (109 mg, 0.875 mmol) was added to a stirred solution of the Troc-protected carbinolamine 63 (93 mg, 0.175 mmol) in THF (1 mL) and aqueous 1N ammonium acetate (1 mL). After 45 min at room temperature TLC revealed complete reaction (70% EtOAc/Petroleum Ether). The mixture was diluted with EtOAc 10 (30 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo. The crude residue was purified by flash chromatography (70% EtOAc/Petroleum Ether) to provide the novel PBD (64, BSD-SJG, **UP2023)** as a white solid (27 mg, 46%): mp °C; <sup>1</sup>H NMR (270 MHz,  $CDCl_3 + CD_3OD$ ) (115,11aS isomer)  $\delta$  8.10 (d, 1H, J = 1.46 Hz), 7.65 (d, 1H, J = 8.79 Hz), 6.86 (d, 1H, J = 8.06 Hz), 5.14-5.10 15 (m, 2H), 4.66 (d, 1H, J = 5.13 Hz), 4.34 (d, 1H, J = 16.12 Hz),4.23 (d, 1H, J = 16.12 Hz), 3.80-3.71 (m, 1H), 3.34 (s, 3H), 3.03-2.86 (m, 1H), 2.65 (d, 1H, J = 16.02 Hz); MS (EI), m/z(relative intensity) (N10-C11 imine form) 339 ( $M^{+}$  + 1, 20), 338  $(M^+, 100), 337 (17), 323 (5), 311 (4), 310 (5), 257 (5), 230$ 20 (4), 229 (13), 211 (4), 203 (4), 202 (8), 184 (8), 183 (4), 103 (5), 82 (17), 81 (4), 80 (5), 76 (6), 75 (16), 74 (5), 55 (4), 53 (4); IR (NUJOL®) 3295 (br), 2923, 2853, 1716, 1615, 1506, 1457, 1377, 1317, 1278, 1238, 1169, 1118, 1063, 999, 895, 818, 751, 718 25  $cm^{-1}$ ; exact mass calcd for  $C_{13}H_{11}N_2OI$  m/e 337.9916, obsd m/e

337.9870.

### Example 2(b): Synthesis of the C8-Benzyl-C7-Methoxy-C2-methlene PBD Monomer SJG-244 (70) (see Figure 7)

### (2S) -N-(4-Benzyloxy-5-methoxy-2-nitrobenzoyl)-2-(tert-butyldimethylsilyloxymethyl)-4-methylidenepyrrolidine (65)

A catalytic amount of DMF (2 drops) was added to a stirred 5 solution of the nitro-acid 1 (0.645 g, 2.13 mmol) and oxalyl chloride (0.23 mL, 0.33 g, 2.60 mmol) in  $CH_2Cl_2$  (40 mL). After 16 h at room temperature the resulting acid chloride solution was added dropwise to a stirred mixture of the amine 58 (0.522 g, 2.30 mmol) and TEA (0.58 g, 0.80 mL, 5.73 mmol) in  $CH_2Cl_2$  (5 mL) 10 at 0°C (ice/acetone) under a nitrogen atmosphere. The reaction mixture was allowed to warm to room temperature and stirred for a further 2.5 h. The mixture was diluted with CH2Cl2 (50 mL), washed with saturated  $NaHCO_3$  (50 mL), saturated  $NH_4Cl$  (50 mL),  $\rm H_{2}O$  (50 mL), brine (50 mL), dried (MgSO<sub>4</sub>), filtered and 15 evaporated in vacuo to give the crude product as a dark orange oil. Purification by flash chromatography (20% EtOAc/Petroleum Ether) isolated the pure amide 65 as a sticky orange oil (0.86 g, 79%):  $[\alpha]^{22}_D = -47.2$  ° (c = 2.79, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) (Rotamers)  $\delta$  7.78 and 7.77 (s x 2, 1H<sub>arom</sub>), 7.48-7.35 (m, 5H<sub>arom</sub>), 20 6.82 and 6.78 (s x 2,  $1H_{arom}$ ), 5.23 and 5.21 (s x 2, 2H,  $PhCH_2O$ ), 5.09-4.83 (m, 2H, NCH<sub>2</sub>C=CH<sub>2</sub>), 4.59-4.49 (m, 1H, NCHCH<sub>2</sub>OTBDMS), 4.03-3.08 (m, 7H, NCHC $H_2$ OTBDMS, NC $H_2$ C=CH<sub>2</sub> and OC $H_3$ ), 2.80-2.56 (m, 2H,  $NCH_2C=CH_2CH_2$ ), 0.89 and 0.79 (s x 2, 9H,  $SiC(CH_3)_3$ ), 0.122, -0.11 and -0.14 (s x 3, 6H, Si( $CH_3$ )<sub>2</sub>); <sup>13</sup>C NMR (67.8 MHz, 25

CDCl<sub>3</sub>) (Rotamers)  $\delta$  166.2 (NC=0), 154.8 and 154.6 ( $C_{quat}$ ), 148.2 and 148.0  $(C_{\text{quat}})$ , 144.1 and 143.2  $(C_{\text{quat}})$ , 137.1  $(C_{\text{quat}})$ , 135.3  $(C_{\text{quat}})$ , 128.8 and 128.5  $(BnC-H_{\text{arom}})$ , 128.2  $(C_{\text{quat}})$ , 127.6  $(BnC-H_{\text{arom}})$  $H_{arom}$ ), 110.1 and 109.2 ( $C-H_{arom}$ ), 109.0 and 108.5 ( $C-H_{arom}$ ), 107.5  $(NCH_2C=CH_2)$ , 71.3  $(PhCH_2O)$ , 63.7  $(NCH_2OTBDMS)$ , 60.2 5  $(NCHCH_2OTBDMS)$ , 58.1 and 56.6  $(OCH_3)$ , 52.8 and 50.5  $(NCH_2C=CH_2)$ , 34.9 and 33.9  $(NCH_2C=CH_2CH_2)$ , 25.8 and 25.7  $(SiC(CH_3)_3)$ , 18.2 (SiC(CH<sub>3</sub>)<sub>3</sub>), -5.4 and -5.6 (Si(CH<sub>3</sub>)<sub>2</sub>); MS (EI), m/z (relative intensity) 512 (M<sup>+</sup>·, 3), 497 (M-CH<sub>3</sub>, 4), 455 (M-<sup>t</sup>Bu, 100), 380 (2), 364 (5), 286  $(M-NCH_2C=CH_2CH_2CHCH_2OTBDMS, 40)$ , 279 (9), 226 10  $(NCH_2C=CH_2CH_2CHCH_2OTBDMS, 5), 168 (10), 149 (27), 91 (PhCH_2, 62),$ 73 (8), 57 (9); IR (NEAT) 3066, 3034, 2953, 2856, 2245, 1644 (NC=O), 1578, 1520, 1454, 1426, 1379, 1335, 1276, 1220, 1186, 1106, 1059, 1016, 910, 836, 815, 779, 734, 697, 655, 614 cm $^{-1}$ .

(2S) -N-(4-Benzyloxy-5-methoxy-2-nitrobenzoyl) -2-(hydroxymethyl) 4-methylidenepyrrolidine (66)

A solution of TBAF (2.10 mL of a 1M solution in THF, 2.10 mmol) was added to the silyl-ether 65 (0.86 g, 1.68 mmol) in THF (20 mL) at 0°C (ice/acetone). The reaction mixture was allowed to warm to room temperature following a colour change (yellow-dark red). After a further 40 min TLC (50% EtOAc/Pet-Ether 40°-60°) revealed the complete disappearance of starting material. Saturated NH<sub>4</sub>Cl (100 mL) was added and the reaction mixture extracted with EtOAc (3 X 40 mL), washed with brine (30 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo to give a dark orange oil which was purified by flash chromatography (60% EtOAc/Petroleum Ether) to provide the pure alcohol 66 as a white

solid (0.64 g, 96%):  $[\alpha]^{19}_{D} = -22.9$  ° (c = 0.20, MeOH); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) (Rotamers)  $\delta$  7.78 and 7.76 (s x 2, 1H<sub>arom</sub>), 7.49-7.33 (m,  $5H_{arom}$ ), 6.91 and 6.82 (s x 2,  $1H_{arom}$ ), 5.22 (s, 2H,  $PhCH_{2}O$ ), 5.10 (m, 1H, OH), 5.03-5.01 (m, 1H,  $NCH_{2}C=CH_{2}$ ), 4.90-4.85 (m, 1H,  $NCH_2C=CH_2$ ), 4.65-4.55 (m, 1H,  $NCH_2OH$ ), 3.99 and 5 3.95 (s x 2, 3H, OC $H_3$ ), 3.90-3.72 (m, 4H, NCHC $H_2$ OH and  $NCH_2C=CH_2$ ), 2.90-2.87 (m, 1H,  $NCH_2C=CH_2CH_2$ ), 2.53-2.47 (m, 1H,  $NCH_2C=CH_2CH_2$ ); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>) (Rotamers)  $\delta$  177.4 (NC=0), 155.1  $(C_{quat})$ , 148.3  $(C_{quat})$ , 142.6  $(C_{quat})$ , 137.0  $(C_{quat})$ , 135.2 ( $C_{\text{quat}}$ ), 128.9, 128.6 and 127.6 (BnC-H<sub>arom</sub>), 109.1 (C-H<sub>arom</sub>), 10 108.5 ( $C-H_{arom}$ ), 108.3 ( $NCH_2C=CH_2$ ), 71.4 ( $PhCH_2O$ ), 65.2 and 63.7  $(NCHCH_2OH)$ , 60.4  $(NCHCH_2OH)$ , 56.8 and 56.7  $(OCH_3)$ , 53.0 and 50.1  $(NCH_2C=CH_2)$ , 35.1 and 34.4  $(NCH_2C=CH_2CH_2)$ ; MS (EI), m/z (relative)intensity) 398 (M+·, 2), 380 (3), 368 (4), 354 (1), 286 (M-NCH<sub>2</sub>C=CH<sub>2</sub>CH<sub>2</sub>CHCH<sub>2</sub>OH, 54), 270 (2), 256 (1), 164 (2), 136 (4), 135 15 (3), 121 (4), 112 (NCH<sub>2</sub>C=CH<sub>2</sub>CH<sub>2</sub>CHCH<sub>2</sub>OH, 3), 91 (PhCH<sub>2</sub>, 100), 82 (3), 69 (4), 65 (6); IR (NUJOL®) 3600-3200 (br, OH), 2923, 2853, 1718, 1663, 1611 (NC=O), 1577, 1517, 1460, 1376, 1332, 1275, 1224, 1176, 1052, 990, 925, 886, 862, 796, 759, 723, 702 615 cm  $^{1}$ ; exact mass calcd for  $C_{21}H_{22}N_{2}O_{6}$  m/e 398.1478, obsd m/e 20 398.1490.

### (2S) -N-(2-Amino-4-benzyloxy-5-methoxybenzoyl) -2-(hydroxymethyl) -4-methylidenepyrrolidine (67)

The nitro-alcohol **66** (0.637 g, 1.60 mmol), SnCl<sub>2</sub> 2H2O (1.81 g, 8.0 mmol) and methanol (36 mL) were heated at reflux and monitored by TLC (90% CHCl<sub>3</sub>/MeOH). After 1 h the MeOH was evaporated in vacuo and the resulting residue cooled (ice), and

treated carefully with saturated NaHCO3 (120 mL). The mixture was diluted with EtOAc (120 mL), and after 16 h stirring at room temperature the inorganic precipitate was removed by filtration through celite. The organic layer was separated, washed with brine (100 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo 5 to give an orange glass. Flash chromatography (EtOAc) afforded the pure amine 67 as a pale yellow glass (0.37 g, 63%):  $[\alpha]^{23}_{D} =$ -42.7 ° (c = 3.7, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.44-7.29 (m,  $5H_{arom}$ ), 6.77 (s,  $1H_{arom}$ ), 6.27 (s,  $1H_{arom}$ ), 5.12 (s, 2H,  $PhCH_2O$ ), 5.06-5.00 (m, 1H, NCH<sub>2</sub>C=CH<sub>2</sub>), 4.99-4.92 (m, 1H, NCH<sub>2</sub>C=CH<sub>2</sub>), 4.63-10 4.53 (m, 1H, NCHCH<sub>2</sub>OH), 4.25-3.60 (m, 10H, NCHCH<sub>2</sub>OH, NCH<sub>2</sub>C=CH<sub>2</sub>,  $OCH_{3}$ , OH and  $NH_{2}$ ), 2.77 (dd, 1H, J = 8.52, 15.85 Hz,  $NCH_2C=CH_2CH_2$ ), 2.43-2.39 (m, 1H,  $NCH_2C=CH_2CH_2$ ); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  171.4 (NC=0), 151.0 ( $C_{quat}$ ), 143.3 ( $C_{quat}$ ), 141.5 ( $C_{quat}$ ), 140.6 ( $C_{\text{quat}}$ ), 136.5 ( $C_{\text{quat}}$ ), 128.6 and 128.0 (BnC-H<sub>arom</sub>), 127.8 .15  $(C_{\text{quat}})$ , 127.1  $(BnC-H_{\text{arom}})$ , 112.5  $(C-H_{\text{arom}})$ , 107.8  $(NCH_2C=CH_2)$ , 103.0 ( $C-H_{arom}$ ), 70.6 ( $PhCH_2O$ ), 65.9 ( $NCHCH_2OH$ ), 60.0 ( $NCHCH_2OH$ ), 57.1 (OCH<sub>3</sub>), 53.3 (NCH<sub>2</sub>C=CH<sub>2</sub>), 34.4 (NCH<sub>2</sub>C=CH<sub>2</sub>CH<sub>2</sub>); MS (EI), m/z(relative intensity) 368 ( $M^+$ , 100), 353 ( $M-CH_3$ , 2), 340 (1), 286 (2), 273 (4), 256  $(M-NCH_2C=CH_2CH_2CH_2CH_2OH, 59)$ , 249 (8), 226 (4), 20 200 (2), 196 (2), 166 (5), 138 (17), 112 ( $NCH_2C=CH_2CH_2CHCH_2OH$ , 39), 91 (PhCH<sub>2</sub>, 70), 82 (5), 65 (5); IR (NEAT) 3600-3000 (br, NH<sub>2</sub> and OH), 3065, 3052, 2932, 2869, 2246, 1668, 1620, 1592, 1513, 1454, 1408, 1264, 1229, 1197, 1176, 1113, 1079, 1002, 909, 733, 698, 645 cm<sup>-1</sup>; exact mass calcd for  $C_{21}H_{24}N_2O_4$  m/e 368.1736, obsd 25 m/e 368.1662.

(2S) -N-[(2-Allyloxycarbonylamino)-4-benzyloxy-5-methoxybenzoyl]2-(hydroxymethyl)-4-methylidenepyrrolidine (68)

A solution of the amino-alcohol 67 (0.33 g, 0.90 mmol) in  $CH_2Cl_2$ (20 mL) was cooled to 0°C (ice/acetone) and treated with pyridine  $(0.14 \ \text{mL}, \ 0.14 \ \text{g}, \ 1.77 \ \text{mmol})$ . A solution of allyl chloroformate 5 (87  $\mu$ L, 99 mg, 0.82 mmol) in  $CH_2Cl_2$  (7 mL) was then added dropwise to the stirred mixture. The reaction mixture was allowed to warm to room temperature and stirred for a further 2.5 h, at which point TLC (EtOAc) revealed complete consumption of amine 67. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (30 mL) 10 and washed with saturated CuSO<sub>4</sub> (40 mL), H<sub>2</sub>O (40 mL), brine (40 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo. crude residue was purified by flash chromatography (80% EtOAc/Petroleum Ether) to afford the pure alloc-amino compound 68 as a white solid (0.34 g, 84%):  $[\alpha]^{22}_{D} = -22.4$  ° (c = 3.4, CHCl<sub>3</sub>); 15  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  8.52 (br s, 1H, NH), 7.82 (br s,  $1H_{arom}$ ), 7.49-7.29 (m,  $5H_{arom}$ ), 6.84 (s,  $1H_{arom}$ ), 6.02-5.88 (m,  $1H_{arom}$ )  $NCO_2CH_2CH=CH_2$ ), 5.39-5.22 (m, 2H,  $NCO_2CH_2CH=CH_2$ ), 5.17 (s, 2H,  $PhCH_2O$ ), 5.01 (br s, 1H,  $NCH_2C=CH_2$ ), 4.94 (br s, 1H,  $NCH_2C=CH_2$ ), 20 4.64-4.59 (m, 3H, NCHCH<sub>2</sub>OH and NCO<sub>2</sub>CH<sub>2</sub>CH=CH<sub>2</sub>), 4.21-3.60 (m, 8H,  $NCHCH_2OH$ ,  $NCH_2C=CH_2$ ,  $OCH_3$  and OH), 2.77 (dd, 1H, J=8.61, 15.94 Hz,  $NCH_2C=CH_2CH_2$ ), 2.46 (d, 1H, J=15.94 Hz,  $NCH_2C=CH_2CH_2$ ); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  171.4 (NC=O<sub>amide</sub>), 153.7 (NC=O<sub>carbamate</sub>), 150.3 (C<sub>quat</sub>), 144.5 (C<sub>quat</sub>), 143.0 (C<sub>quat</sub>), 136.2 (C<sub>quat</sub>), 132.4  $(NCO_2CH_2CH=CH_2)$ , 131.3  $(C_{quat})$ , 128.6, 128.1, and 127.7  $(BnC-CH_2CH=CH_2)$ 25  $H_{arom}$ ), 118.1 (NCO<sub>2</sub>CH<sub>2</sub>CH=CH<sub>2</sub>), 111.1 (C- $H_{arom}$ ), 108.1 (NCH<sub>2</sub>C=CH<sub>2</sub>), 106.5 ( $C-H_{arom}$ ), 70.7 ( $PhCH_2O$ ), 65.8 ( $NCO_2CH_2CH=CH_2$ ), 65.5  $(NCHCH_2OH)$ , 59.9  $(NCHCH_2OH)$ , 56.7  $(OCH_3)$ , 54.0  $(NCH_2C=CH_2)$ , 34.1

 $(NCH_2C=CH_2CH_2); MS (EI), m/z (relative intensity) 452 (M^+, 38),$  395  $(M-OC_3H_5, 4), 394 (10), 340 (M-NCH_2C=CH_2CH_2CHCH_2OH, 20), 298 (7), 282 (22), 255 (8), 206 (2), 192 (2), 163 (3), 136 (3), 114 (6), 112 <math>(NCH_2C=CH_2CH_2CHCH_2OH, 12), 91 (PhCH_2, 100), 82 (10), 65 (4), 57 (0C_3H_5, 7); IR (NUJOL®) 3600-2000 (br, OH), 3335, 3242, 2922, 2854, 1724, 1614, 1537, 1463, 1407, 1378, 1349, 1280, 1214, 1178, 1117, 1054, 1028, 995, 947, 908, 892, 853, 821, 768, 735, 697, 629, 601, 514 cm<sup>-1</sup>; exact mass calcd for <math>C_{25}H_{28}N_2O_6$  m/e 452.1947, obsd m/e 452.1923.

(11s,11as)-10-Allyloxycarbonyl-8-benzyloxy-11-hydroxy-7-methoxy2-methylidene-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1c][1,4]benzodiazepin-5-one (69)

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A solution of DMSO (0.18 mL, 0.20 g, 2.56 mmol) in  $CH_2Cl_2$  (4 mL) was added dropwise over 30 min to a solution of oxalyl chloride (0.63 mL of a 2.0 M solution in  $CH_2Cl_2$ , 1.26 mmol) at -45°C (dry ice/ $CH_3CN$ ) under a nitrogen atmosphere. After stirring at -45°C for 30 min, a solution of the alcohol 68 (0.42 g, 0.93 mmol) dissolved in  $CH_2Cl_2$  (8 mL) was added dropwise over 35 min at -45°C. After 45 min at -45°C, the mixture was treated dropwise with TEA (0.50 mL, 0.36 g, 3.56 mmol) in  $CH_2Cl_2$  (4 mL) over 30 min at -45°C. After 35 min, the reaction mixture was allowed to warm to room temperature and was diluted with  $CH_2Cl_2$  (30 mL), washed with 1N HCl (20 mL),  $H_2O$  (20 mL), brine (30 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo. TLC (80% EtOAc/Petroleum Ether) of the crude material revealed sufficient product formation and a trace of unoxidised starting material.

Purification by flash chromatography (50% EtOAc/Petroleum Ether)

furnished the protected carbinolamine 69 as white glass (0.172 g, 41%):  ${}^{1}H$  NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.48-7.27 (m,  $5H_{arom}$ ), 7.25 (s,  $1H_{arom}$ ), 6.74 (br s,  $1H_{arcm}$ ), 5.65-5.53 (m, 1H,  $NCO_2CH_2CH=CH_2$ ), 5.56 (d, 1H, J = 9.89 Hz, NCHCHOH), 5.22-5.04 ( $m_7$ -6H, NCH<sub>2</sub>C=CH<sub>2</sub>,  $NCO_2CH_2CH=CH_2$  and  $PhCH_2O)$ , 4.64-4.42 (m, 3H,  $NCO_2CH_2CH=CH_2$  and OH), 4.28 (d, 1H, J = 15.94 Hz,  $NCH_2C=CH_2$ ), 4.09 (d, 1H, J =15.94 Hz,  $NCH_2C=CH_2$ ), 3.92 (s, 3H,  $OCH_3$ ), 3.62 (t, 1H, J=8.79Hz, NCHCHOH), 2.90 (dd, 1H, J = 8.97, 16.03 Hz,  $NCH_2C=CH_2CH_2$ ), 2.67 (d, 1H, J = 16.03 Hz,  $NCH_2C=CH_2CH_2$ ); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  166.8 (NC=O<sub>amide</sub>), 156.0 (NC=O<sub>carbamate</sub>), 150.1 (C<sub>quat</sub>), 10 149.0 ( $C_{\text{quat}}$ ), 141.8 ( $C_{\text{quat}}$ ), 136.1 ( $C_{\text{quat}}$ ), 131.8 ( $NCO_2CH_2CH=CH_2$ ), 128.6, 128.1 and 127.3 ( $BnC-H_{arom}$ ), 125.6 ( $C_{quat}$ ), 118.0  $(NCO_2CH_2CH=CH_2)$ , 114.6  $(C-H_{arom})$ , 110.6  $(C-H_{arom})$ , 109.8  $(NCH_2C=CH_2)$ , 85.8  $(NCH_2CHOH)$ , 71.0  $(Ph_2CH_2O)$ , 66.7  $(NCO_2CH_2CH=CH_2)$ , 15 59.8 (NCHCHOH), 56.2 (OCH<sub>3</sub>), 50.7 (NCH<sub>2</sub>C=CH<sub>2</sub>), 35.0  $(NCH_2C=CH_2CH_2)$ ; MS (EI), m/z (relative intensity) 450 (M<sup>+</sup>·, 24), 422 (1), 392 (1), 364 (1), 348 (3), 340 (12), 298 (6), 282 (8), 257 (2), 229 (2), 192 (3), 178 (2), 164 (4), 136 (3), 110 (3), 91 (PhCH<sub>2</sub>, 100), 82 (17), 65 (7); IR (NUJOL®) 3600-2500 (br, OH), 2923, 2854, 1711, 1619, 1601, 1513, 1463, 1405, 1377, 1300, 1278, 20 1202, 1119, 1045, 993, 956, 909, 790, 768, 724, 697, 637 cm $^{-1}$ ; exact mass calcd for  $C_{25}H_{26}N_2O_6$  m/e 450.1791, obsd m/e 450.1790.

(11s,11as)-10-Allyloxycarbonyl-8-benzyloxy-11-hydroxy-7-methoxy-2-methylidene-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (69)

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A solution of the alcohol **68** (0.32 g, 0.71 mmol) in  $CH_2Cl_2/CH_3CN$  (30 mL, 3:1) was treated with 4 Å powdered molecular sieves (0.2

g) and NMO (126 mg, 1.08 mmol). After 15 min stirring at room temperature, TPAP (12.6 mg, 35.9 µmol) was added and stirring continued for a further 1 h 20 min at which point TLC (80% EtOAc/Petroleum Ether) revealed product formation along with some unoxidised starting material. The mixture was then treated with 5 a further quantity of NMO (126 mg, 1.08 mmol) and TPAP (12.6 mg, 35.9 µmol), and allowed to stir for a further 0.5 h after which time TLC revealed reaction completion. The mixture was evaporated in vacuo onto silica and subjected to flash chromatography (50% EtOAc/Petroleum Ether) to provide the 10 protected carbinolamine 69 as a white glass (153 mg, 48%):  $\left[\alpha\right]^{23}$ <sub>D</sub> = +129.8 ° (c = 1.5, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.48-7.27  $(m, 5H_{arom}), 7.25 (s, 1H_{arom}), 6.74 (br s, 1H_{arom}), 5.65-5.53 (m,$ 1H,  $NCO_2CH_2CH=CH_2$ ), 5.56 (d, 1H, J = 9.89 Hz, NCHCHOH), 5.22-5.04 (m, 6H, NCH<sub>2</sub>C=CH<sub>2</sub>, NCO<sub>2</sub>CH<sub>2</sub>CH=CH<sub>2</sub> and PhCH<sub>2</sub>O), 4.64-4.42 (m, 3H,15  $NCO_2CH_2CH=CH_2$  and OH), 4.28 (d, 1H, J=15.94 Hz,  $NCH_2C=CH_2$ ), 4.09 (d, 1H, J = 15.94 Hz,  $NCH_2C=CH_2$ ), 3.92 (s, 3H,  $OCH_3$ ), 3.62 (t, 1H, J = 8.79 Hz, NCHCHOH), 2.90 (dd, 1H, J = 8.97, 16.03 Hz,  $NCH_2C=CH_2CH_2$ ), 2.67 (d, 1H, J=16.03 Hz,  $NCH_2C=CH_2CH_2$ ); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>) δ 166.8 (NC=O<sub>amide</sub>), 156.0 (NC=O<sub>carbamate</sub>), 150.1 20  $(C_{\text{quat}})$ , 149.0  $(C_{\text{quat}})$ , 141.8  $(C_{\text{quat}})$ , 136.1  $(C_{\text{quat}})$ , 131.8  $(NCO_2CH_2CH=CH_2)$ , 128.6, 128.1 and 127.3  $(BnC-H_{arom})$ , 125.6  $(C_{quat})$ , 118.0  $(NCO_2CH_2CH=CH_2)$ , 114.6  $(C-H_{arom})$ , 110.6  $(C-H_{arom})$ , 109.8  $(NCH_2C=CH_2)$ , 85.8 (NCHCHOH), 71.0  $(PhCH_2O)$ , 66.7  $(NCO_2CH_2CH=CH_2)$ , 59.8 (NCHCHOH), 56.2 (OCH<sub>3</sub>), 50.7 (NCH<sub>2</sub>C=CH<sub>2</sub>), 35.0 25 (NCH<sub>2</sub>C=CH<sub>2</sub>CH<sub>2</sub>); MS (EI), m/z (relative intensity) 450 (M<sup>+</sup>·, 24),422 (1), 392 (1), 364 (1), 348 (3), 340 (12), 298 (6), 282 (8), 257 (2), 229 (2), 192 (3), 178 (2), 164 (4), 136 (3), 110 (3), 91

 $(PhCH_2, 100), 82 (17), 65 (7); IR (NUJOL<sup>®</sup>) 3600-2500 (br, OH),$ 2923, 2854, 1711, 1619, 1601, 1513, 1463, 1405, 1377, 1300, 1278, 1202, 1119, 1045, 993, 956, 909, 790, 768, 724, 697, 637 cm<sup>-1</sup>; exact mass calcd for  $C_{25}H_{26}N_2O_6$  m/e 450.1791, obsd m/e 450.1790.

5 (11aS) -8-Benzyloxy-7-methoxy-2-methylidene-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (70, SJG-244)

A catalytic amount of tetrakis(triphenylphosphine)palladium (12.0 mg, 10.4 μmol) was added to a stirred solution of the Allocprotected carbinolamine 69 (0.18 g, 0.40 mmol),

- triphenylphosphine (5.25 mg, 20 µmol) and pyrrolidine (29 mg, 10 0.41 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (15 mL). After 2 h stirring at room temperature under a nitrogen atmosphere, TLC (98% CHCl<sub>3</sub>/MeOH) revealed the complete consumption of starting material. The solvent was evaporated in vacuo and the crude residue was
- purified by flash chromatography (60% EtOAc/Petroleum Ether) to 15 afford 70 (SJG-244) as a white glass (116 mg, 83%) which was repeatedly evaporated in vacuo with CHCl3 in an attempt to provide the N10-C11 imine form:  $[\alpha]^{22}_{D} = +754.2$  ° (c = 0.54, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) (mainly imine, plus trace of
- carbinolamine form)  $\delta$  7.70-7.30 (m, 7H, HC=N and  $6H_{arom}$ ), 6.84  $(s, 1H_{arom}), 5.25-5.13$  (m, 4H, NCH<sub>2</sub>C=CH<sub>2</sub> and PhCH<sub>2</sub>O), 4.42 (br s,

20

- 2H,  $NCH_2C=CH_2$ ), 3.95 (s, 3H,  $OCH_3$ ), 3.88-3.66 (m, 1H, NCHHC=N), 3.09 (dd, 1H, J = 8.98, 16.12 Hz, NCH<sub>2</sub>C=CH<sub>2</sub>CH<sub>2</sub>), 2.94-2.87 (m,
- 1H,  $NCH_2C=CH_2CH_2$ ); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  164.7 (NC=0), 162.6
- (HC=N), 150.6  $(C_{quat})$ , 148.1  $(C_{quat})$ , 141.6  $(C_{quat})$ , 140.5  $(C_{quat})$ , 25
  - 136.1 ( $C_{\text{quat}}$ ), 132.0, 128.7, 128.6, 128.1 and 127.3 (BnC-H<sub>arom</sub>),
  - 120.1  $(C_{quat})$ , 111.5  $(C-H_{arom})$ , 111.2  $(C-H_{arom})$ , 109.4  $(NCH_2C=CH_2)$ ,

70.8 (PhCH<sub>2</sub>O), 56.2 (OCH<sub>3</sub>), 53.7 (NCHHC=N), 51.3 (NCH<sub>2</sub>C=CH<sub>2</sub>),
35.4 (NCH<sub>2</sub>C=CH<sub>2</sub>CH<sub>2</sub>); MS (EI), m/z (relative intensity) (imine
form) 348 (M<sup>+</sup>, 100), 333 (M-CH<sub>3</sub>, 42), 319 (3), 269 (5), 257 (M-PhCH<sub>2</sub>, 25), 241 (11), 229 (56), 227 (11), 213 (5), 186 (4), 156 (6), 136 (22), 122 (4), 91 (PhCH<sub>2</sub>, 85), 82 (5), 65 (22); IR
(NUJOL®) 3318 (br, OH of carbinolamine form), 2923, 2853, 1722,
1668, 1600, 1557, 1504, 1462, 1377, 1261, 1216, 1120, 1003, 892,
789, 722, 695, 623, 542 cm<sup>-1</sup>; exact mass calcd for C<sub>21</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub> m/e
348.1474, obsd m/e 348.1469.

#### Example 2(c): Synthesis of MMY-SJG (74, UP2064) (see Figure 8)

(25)-N-[(2-Allyloxycarbonylamino)-4,5-dimethoxybenzoy1]-2-(tert-butyldimethylsilyloxymethyl)-4-methylidinepyrrolidine (71)

Potassium tert-butoxide (21.2 mL of a 0.5 M solution in THF, 10.6 mmol) was added dropwise to a suspension of

15 methyltriphenylphosphonium bromide (3.78 g, 10.6 mmol) in THF (11 mL) at 0°C (ice/acetone) under nitrogen. After stirring for 2 h at 0°C, a solution of the ketone 38 (Example 1(e)) (2.0 g, 4.07 mmol) in THF (7 mL) was added dropwise and the mixture allowed to warm to room temperature. After stirring for a

20 further 45 min the reaction mixture was diluted with EtOAc (60 mL) and water (60 mL). The organic layer was separated, washed with brine, dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo to give a dark oil. Purification by flash chromatography

(20% EtOAc/Petroleum Ether) isolated the pure olefin 71 as a transparent oil (1.71 g, 86%):  $[\alpha]^{22}_{p} = -44.55$  ° (c = 0.20,  $CHCl_3$ ); <sup>1</sup>H NMR (270 MHz,  $CDCl_3$ ) (Rotamers)  $\delta$  8.85 (br s, 1H), 7.86 (s, 1H), 6.82 (s, 1H), 6.03-5.89 (m, 1H), 5.35 (ddd, 1H, J =17.22, 3.11, 1.47 Hz), 5.24 (ddd, 1H, J = 10.44, 2.75, 1.28 Hz), 5 4.99-4.92 (m, 2H), 4.70-4.57 (m, 3H), 4.23-3.57 (m, 10H), 2.72-2.68 (m, 2H), 0.96-0.85 (m, 9H), 0.09--0.03 (m, 6H);  $^{13}$ C NMR  $(67.8 \text{ MHz}, \text{CDCl}_3)$  (Rotamers)  $\delta$  168.7, 153.6, 150.9, 143.6, 132.5, 132.2, 118.1, 115.3, 110.6, 107.1, 104.3, 65.7, 63.6, 56.3, 56.0, 10 33.1, 25.8, 18.1, -5.5 and -5.6; MS (EI), m/z (relative intensity) 492  $(M^{+} + 2, 7)$ , 491  $(M^{+} + 1, 20)$ , 490  $(M^{+}, 50)$ , 475 (4), 435 (10), 447 (3), 434 (29), 433 (94), 376 (4), 375 (13), 348 (5), 333 (11), 332 (6), 294 (3), 265 (16), 264 (100), 227 (8), 226 (24), 224 (5), 223 (18), 220 (15), 210 (4), 208 (5), 207 15 (13), 206 (96), 192 (7), 180 (18), 179 (25), 170 (21), 169 (8), 168 (28), 164 (13), 152 (7), 150 (13), 136 (10), 108 (5), 96 (5), 95 (12), 94 (7), 89 (8), 82 (25), 75 (20), 73 (30), 59 (7), 58 (5), 57 (41), 56 (7), 55 (4); IR (NEAT) 3324 (br, NH), 3082, 2953, 2930, 2857, 1732, 1600, 1523, 1490, 1464, 1419, 1397, 1360, 20 1333, 1287, 1259, 1228, 1203, 1172, 1115, 1039, 1004, 939, 837, 814, 777 666 cm<sup>-1</sup>.

#### (2S) -N-[(2-Allyloxycarbonylamino) -4,5-dimethoxybenzoyl]-2-(hydroxymethyl)-4-methylidinepyrrolidine (72)

A solution of TBAF (4.29 mL of a 1M solution in THF, 4.29 mmol)

was added to the silyl-ether 71 (1.68 g, 3.43 mmol) in THF (45 mL) at 0°C (ice/acetone). The reaction mixture was allowed to warm to room temperature and after 1 h TLC (50% EtOAc/Pet-Ether

40°- 60°) revealed the complete disappearance of starting material. Saturated  $NH_4Cl$  (110 mL) was added and the reaction mixture extracted with EtOAc (3 X 50 mL), washed with brine (100 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo to give a dark orange oil. Purification by flash chromatography (99% 5 CHCl<sub>3</sub>/MeOH) provided the pure alcohol 72 as a white solid (1.15 g, 89%):  $[\alpha]^{21}_{D} = -13.17$  ° (c = 0.15, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz,  $CDCl_3$ )  $\delta$  8.59 (br s, 1H), 7.69 (s, 1H), 6.82 (s, 1H), 6.03-5.89 (m, 1H), 5.35 (ddd, 1H, J = 17.22, 3.11, 1.65 Hz), 5.24 (ddd, 1H, J = 10.44, 2.75, 1.28 Hz), 5.02-4.94 (m, 2H), 4.66-4.62 (m, 3H), 10 4.23-3.57 (m, 11H), 2.77 (dd, 1H, J = 15.94, 8.42 Hz), 2.48 (d, 1H, J = 15.94 Hz); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  170.3, 153.8, 151.0, 144.2, 143.1, 132.5, 131.2, 118.1, 115.9, 110.4, 108.1, 104.9, 65.8, 65.1, 59.8, 56.4, 56.0, 54.2, 34.1; MS (EI), m/z (relative intensity) 378 ( $M^{+}$  + 2, 3), 377 ( $M^{+}$  + 1, 14), 376 15  $(M^+, 51), 319 (3), 265 (10), 264 (62), 263 (4), 259 (8), 224$ (4), 223 (18), 220 (17), 208 (5), 207 (14), 206 (100), 192 (8), 190 (5), 180 (27), 179 (29), 178 (4), 164 (23), 163 (4), 152 (12), 151 (6), 150 (19), 137 (5), 136 (22), 135 (6), 125 (6), 120 20 (6), 113 (6), 112 (31), 109 (6), 108 (11), 95 (4), 94 (9), 82 (28), 80 (8), 67 (5), 57 (5), 54 (7), 53 (7); IR (NUJOL®) 3341 and 3245 (br, OH and NH), 3115, 2918, 2850, 1727, 1616, 1540, 1464, 1399, 1378, 1351, 1283, 1264, 1205, 1179, 1117, 1055, 1040, 996, 946, 909, 894, 855, 823, 768, 754, 722, 696, 623,  $602 \text{cm}^{-1}$ ; exact mass calcd for  $C_{19}H_{24}N_2O_6$  m/e 376.1634, obsd m/e 376.1614. 25

(11s,11as)-10-Allyloxycarbonyl-7,8-dimethoxy-11-hydroxy-2-methylidene-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (73)

A solution of DMSO (0.75 mL, 0.82 g, 10.5 mmol) in  $CH_2Cl_2$  (27 mL) was added dropwise over 38 min to a solution of oxalyl chloride 5 (2.64 mL of a 2.0 M solution in  $CH_2Cl_2$ , 5.27 mmol) at -45°C (lig.N<sub>2</sub>/Chlorobenzene) under a nitrogen atmosphere. After stirring at -45°C for 1 h, a solution of the alcohol 72 (1.10 g, 2.93 mmol) in  $CH_2Cl_2$  (27 mL) was added dropwise over 1 h at -45°C. After 1 h at -45°C, the mixture was treated dropwise with 10 a solution of TEA (1.71 mL, 1.24 g, 12.29 mmol) in  $CH_2Cl_2$  (15 mL) over 40 min at -45°C. After a further 30 min, the reaction mixture was allowed to warm to room temperature and was diluted with  $CH_2Cl_2$  (50 mL), washed with 1N HCl (50 mL),  $H_2O$  (50 mL), brine (50 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo. 15 TLC (80% EtOAc/Petroleum Ether) of the crude material revealed reaction completion. Purification by flash chromatography (60% EtOAc/Petroleum Ether) furnished the protected carbinolamine 73 as a white glass (0.45 g, 41%):  $[\alpha]^{22}_{D} = +236.51$  ° (c = 0.14,  $CHCl_3$ ); <sup>1</sup>H NMR (270 MHz,  $CDCl_3$ )  $\delta$  7.23 (s, 1H), 6.69 (s, 1H), 20 5.83-5.81 (m, 1H), 5.60-5.58 (m, 1H), 5.34-5.23 (m, 4H), 4.74-4.66 (m, 1H), 4.50-4.40 (m, 1H), 4.30 (d, 1H, J = 15.94 Hz), 4.15(d, 1H, J = 15.93 Hz), 3.96-3.86 (m, 7H), 3.65 (t, 1H, J = 8.61Hz), 2.92 (dd, 1H, 16.21, 9.07 Hz), 2.70 (d, 1H, J = 15.94 Hz); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>) δ 166.7, 156.0, 150.8, 148.4, 141.8, 25 131.7, 128.5, 125.2, 118.1, 112.4, 110.3, 109.8, 85.9, 66.8, 59.6, 56.3, 56.1, 50.7, 35.0; MS (EI), m/z (relative intensity)  $376 (M^{+} + 2, 6), 375 (M^{+} + 1, 22), 374 (M^{+}, 100), 346 (5), 293$ 

(8), 288 (10), 271 (5), 265 (11), 264 (67), 248 (5), 237 (5), 223 (10), 220 (9), 209 (6), 208 (42), 207 (14), 206 (70), 192 (7), 190 (5), 180 (17), 179 (16), 165 (8), 164 (15), 153 (5), 152 (10), 150 (12), 149 (7), 137 (6), 136 (10), 135 (5), 125 (8), 110 (8), 108 (5), 94 (5), 83 (5), 82 (59), 80 (7), ; IR (CHCl<sub>3</sub>) 3275 (br, OH), 3075, 2936, 2851, 1706, 1624, 1604, 1516, 1457, 1436, 1403, 1368, 1312, 1301, 1278, 1262, 1218, 1119, 1074, 1045, 940, 916, 893, 867, 851, 666, 637 cm<sup>-1</sup>; exact mass calcd for  $C_{19}H_{22}N_2O_6$  m/e 374.1478, obsd m/e 374.1687.

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(11aS) -7,8-Dimethoxy-2-methylidene-1,2,3,11a-tetrahydro-5H-10 pyrrolo[2,1-c][1,4]benzodiazepin-5-one (74, UP2064, MMY-SJG) A catalytic amount of tetrakis(triphenylphosphine)palladium (32.4 mg, 28.1 µmol) was added to a stirred solution of the Allocprotected carbinolamine 73 (0.42 g, 1.12 mmol), triphenylphosphine (14.7 mg, 56.2 µmol) and pyrrolidine (83.9 mg, 15 1.18 mmol) in  $CH_2Cl_2$  (55 mL). After 2.5 h stirring at room temperature under a nitrogen atmosphere, TLC (95% CHCl3/MeOH) revealed the complete consumption of starting material. The solvent was evaporated in vacuo and the crude residue was purified by flash chromatography (CHCl3) to afford the novel PBD 20 (74, MMY-SJG, UP2064) as a yellow oil which was repeatedly evaporated in vacuo with CHCl3 in order to provide the N10-C11 imine form (259 mg, 85%):  $[\alpha]^{22}_{D} = +583.14$  ° (c = 1.42, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.69 (d, 1H, J = 4.39 Hz), 7.51 (s, 1H), 6.82 (s, 1H), 5.21-5.17 (m, 2H), 4.44-4.23 (m, 2H), 3.96-3.81 (m, 25 7H), 3.17-3.08 (m, 1H), 2.95 (d, 1H, J = 14.29 Hz);  $^{13}$ C NMR (67.8) MHz, CDCl<sub>3</sub>) δ 164.7, 162.6, 151.5, 147.6, 141.6, 140.8, 119.8,

111.2, 109.4, 109.4, 56.2, 56.1, 53.8, 51.4, 35.5; MS (EI), m/z (relative intensity) 273 (M<sup>+</sup>· + 1, 16), 272 (M<sup>+</sup>·, 100), 271 (35), 270 (9), 255 (5), 243 (7), 241 (7), 230 (6), 228 (6), 226 (5), 212 (3), 192 (4), 191 (16), 165 (4), 164 (19), 163 (4), 136 (22), 93 (6), 82 (7), 80 (3), 53 (3); IR (NEAT) 3312 (br), 3083, 2936, 2843, 1624, 1603, 1505, 1434, 1380, 1264, 1217, 1180, 1130, 1096, 1069, 1007, 935, 895, 837, 786, 696, 666, 594, 542 cm<sup>-1</sup>; exact mass calcd for  $C_{15}H_{16}N_2O_3$  m/e 272.1161, obsd m/e 272.1154.

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## Example 2(d): Synthesis of the PBD Dimer SJG-136 (UP2001) (see 10 Figure 9)

(2s)-1,1'-[[(Propane-1,3-diyl)dioxy]bis[(2-nitro-5-methoxy-1,4-phenylene)carbonyl]]bis[2-(tert-butyldimethylsilyloxymethyl)-4-methylidenepyrrolidine] (75)

A catalytic amount of DMF (2 drops) was added to a solution of
the dimer acid 44 (0.66 g, 1.42 mmol) and oxalyl chloride
(0.31 mL, 0.45 g, 3.55 mmol) in THF (12 mL). The reaction
mixture was stirred for 16 h under nitrogen, concentrated in
vacuo, and redissolved in THF (10 mL). The resulting solution of
bis-acid chloride was added dropwise to the amine 58 (0.65 g,
20 2.86 mmol), H<sub>2</sub>O (0.84 mL) and TEA (0.83 mL, 0.60 g, 5.93 mmol) in
THF (2 mL) at 0°C (ice/acetone) under nitrogen. The reaction
mixture was allowed to warm to room temperature and stirred for a
further 2 h at which time TLC (EtOAc) revealed reaction

completion. After removal of the THF by evaporation in vacuo, the residue was partitioned between  $\rm H_2O$  (100 mL) and EtOAc (100 mL). The aqueous layer was washed with EtOAc (3 X 50 mL), and the combined organic layers washed with saturated NH4Cl (100 mL), brine (100 mL), dried (MgSO<sub>4</sub>), filtered and 5 concentrated in vacuo to give the crude product as a dark orange Purification by flash chromatography (50% EtOAc/Petroleum Ether) afforded the pure amide 75 as a pale yellow glass (0.93 g, 74%):  $[\alpha]^{21}_{D} = -51.1$  ° (c = 0.08, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) (Rotamers)  $\delta$  7.77 and 7.74 (s x 2,  $2H_{arom}$ ), 6.81 and 6.76 (s x 2, 10  $2H_{arom}$ ), 5.09-4.83 (m, 4H,  $NCH_2C=CH_2$ ), 4.60 (m, 2H,  $NCH_2OTBDMS$ ), 4.35-4.31 (m, 4H, OC $H_2$ CH $_2$ CH $_2$ C), 4.08-3.74 (m, 14H, NCHC $H_2$ OTBDMS,  $NCH_2C=CH_2$  and  $OCH_3$ ), 2.72-2.45 (m, 6H,  $NCH_2C=CH_2CH_2$  and  $OCH_2CH_2CH_2O$ ), 0.91 and 0.79 (s x 2, 18H,  $SiC(CH_3)_3$ ), 0.09, -0.09, and -0.12 (s x 3, 12H, Si(CH<sub>3</sub>)<sub>2</sub>); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>) 15 (Rotamers)  $\delta$  166.2 (NC=O), 154.7 and 154.5 ( $\textit{C}_{\texttt{quat}}$ ), 148.4 and 148.2 ( $C_{\text{quat}}$ ), 144.1 and 143.2 ( $C_{\text{quat}}$ ), 137.2 ( $C_{\text{quat}}$ ), 128.2 and 127.4 ( $C_{\text{quat}}$ ), 110.1 and 108.6 ( $C_{\text{Harom}}$ ), 109.1 and 108.3 ( $C_{\text{Harom}}$ )  $H_{arom}$ ), 107.5 (NCH<sub>2</sub>C=CH<sub>2</sub>), 65.7 and 65.5 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 63.9 and 62.6 (NCHCH2OTBDMS), 60.2 (NCHCH2OTBDMS), 58.1 and 56.6 (OCH3), 20 52.8 and 50.5 ( $NCH_2C=CH_2$ ), 35.0 and 33.9 ( $NCH_2C=CH_2CH_2$ ), 30.8 and 28.6 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 25.8 and 25.7 (SiC(CH<sub>3</sub>)<sub>3</sub>), 18.2 (SiC(CH<sub>3</sub>)<sub>3</sub>), -5.5 and -5.6 (Si( $CH_3$ )<sub>2</sub>); MS (EI), m/z (relative intensity) 885  $(M^{+}, 7)$ , 828  $(M^{-t}Bu, 100)$ , 740  $(M^{-t}CH_2OTBDMS, 20)$ , 603 (3), 479 (26), 391 (27), 385 (25), 301 (7), 365 (10), 310 (14), 226 (8), 25 222 (13), 170 (21), 168 (61), 82 (39), 75 (92); IR (NUJOL®) 2923, 2853, 2360, 1647, 1587, 1523 (NO<sub>2</sub>), 1461, 1429, 1371, 1336 (NO<sub>2</sub>), 1277, 1217, 1114, 1061, 1021, 891, 836 772, 739 cm $^{-1}$ .

(2s)-1,1'-[[(Propane-1,3-diyl)dioxy]bis[(2-nitro-5-methoxy-1,4-phenylene)carbonyl]]bis[2-(hydroxymethyl)-4-methylidenepyrrolidine] (76)

A solution of TBAF (3.98 mL of a 1M solution in THF, 3.98 mmol) was added to the bis-silyl ether 75 (1.41 g, 1.59 mmol) in THF 5 (35 mL) at 0°C (ice/acetone). The reaction mixture was allowed to warm to room temperature and after a further 30 min saturated NH<sub>4</sub>Cl (120 mL) was added. The aqueous solution was extracted with EtOAc (3 X 80 mL), washed with brine (80 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo to give a dark orange oil which 10 was purified by flash chromatography (97% CHCl3/MeOH) to provide the pure diol **76** as a light orange solid (0.98 g, 94%):  $[\alpha]^{19}_{D}$  = -31.9 ° (c = 0.09, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) (Rotamers)  $\delta$ 7.75 and 7.71 (s x 2,  $2H_{arom}$ ), 6.96 and 6.84 (s x 2,  $2H_{arom}$ ), 5.08, 5.02 and 4.88 (br s x 3, 4H,  $NCH_2C=CH_2$ ), 4.61-4.50 (m, 2H, 15  $NCHCH_2OH)$ , 4.35-4.33 (m, 4H,  $OCH_2CH_2CH_2O$ ), 4.02-3.65 (m, 14H,  $NCHCH_2OH$ ,  $NCH_2C=CH_2$  and  $OCH_3$ ), 2.88-2.43 (m, 6H,  $NCH_2C=CH_2CH_2$  and  $OCH_2CH_2CH_2O$ ); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>) (Rotamers)  $\delta$  167.9 and 166.9 (NC=0), 154.9 and 154.3 ( $C_{quat}$ ), 148.4 and 148.2 ( $C_{quat}$ ), 143.3 and 142.6 ( $C_{\text{quat}}$ ), 137.2 and 137.0 ( $C_{\text{quat}}$ ), 127.6 and 127.3 20  $(C_{\text{quat}})$ , 109.1  $(C-H_{\text{arom}})$ , 108.4  $(NCH_2C=CH_2)$ , 108.2  $(C-H_{\text{arom}})$ , 65.6 and 65.4  $(OCH_2CH_2CH_2O)$ , 64.5 and 63.3  $(NCHCH_2OH)$ , 60.5 and 60.0 (NCHCH<sub>2</sub>OH), 56.8 and 56.7 (OCH<sub>3</sub>), 52.9 (NCH<sub>2</sub>C=CH<sub>2</sub>), 35.0 and 34.3 (NCH<sub>2</sub>C=CH<sub>2</sub>CH<sub>2</sub>), 29.6 and 28.6 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O); MS (FAB) (Relative Intensity) 657  $(M^{+} + 1, 10)$ , 639 (M-OH, 2), 612 (1), 544 (M-OH, 2)25 NCH<sub>2</sub>CCH<sub>2</sub>CH<sub>2</sub>CHCH<sub>2</sub>OH, 4), 539 (1), 449 (16), 433(9), 404 (8), 236 (32), 166 (65), 151 (81), 112 (82), 82 (100); IR (NUJOL®) 3600-3200 (br, OH), 2923, 2853, 2360, 1618, 1582, 1522 (NO<sub>2</sub>), 1459,

1408, 1375, 1335  $(NO_2)$ , 1278, 1218, 1061, 908, 810, 757 cm<sup>-1</sup>.

(2S)-1,1'-[[(Propane-1,3-diyl)dioxy]bis[(2-amino-5-methoxy-1,4-phenylene)carbonyl]]bis[2-(hydroxymethyl)-4-methylidenepyrrolidine] (77)

A mixture of the diol 76 (0.98 g, 1.49 mmol) and  $SnCl_2.2H_2O$  (3.36 5 q, 14.9 mmol) in MeOH (35 mL) was heated at reflux and the progress of the reaction monitored by TLC (90% CHCl3/MeOH). After 45 min, the MeOH was evaporated in vacuo and the resulting residue was cooled (ice), and treated carefully with saturated NaHCO3 (120 mL). The mixture was diluted with EtOAc (120 mL), 10 and after 16 h stirring at room temperature the inorganic precipitate was removed by filtration through celite. organic layer was separated, washed with brine (100 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo to give a brown solid. Flash chromatography (95% CHCl<sub>3</sub>/MeOH) afforded the pure bis-amine 15 77 as an orange solid (0.54 g, 61%):  $[\alpha]_{D}^{19} = -31.8$  ° (c = 0.30,  $CHCl_3$ ); <sup>1</sup>H NMR (270 MHz,  $CDCl_3$ )  $\delta$  6.74 (s,  $2H_{arom}$ ), 6.32 (s,  $2H_{arom}$ ), 5.00 (br s, 2H, NCH<sub>2</sub>C=CH<sub>2</sub>), 4.93 (br s, 2H, NCH<sub>2</sub>C=CH<sub>2</sub>), 4.54 (br s, 2H, NCHCH<sub>2</sub>OH), 4.24-4.14 (m, 4H, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 3.98-3.50 (m, 14H, NCHCH<sub>2</sub>OH, NCH<sub>2</sub>C=CH<sub>2</sub> and OCH<sub>3</sub>), 2.76 (dd, 2H, J =8.61, 15.91 Hz,  $NCH_2C=CH_2CH_2$ ), 2.46-2.41 (m, 2H,  $NCH_2C=CH_2CH_2$ ), 2.33-2.28 (m, 2H,  $OCH_2CH_2CH_2O$ ); <sup>13</sup>C NMR (67.8 MHz,  $CDCl_3$ )  $\delta$  171.0 (NC=0), 151.0 ( $C_{\rm quat}$ ), 143.5 ( $C_{\rm quat}$ ), 141.3 ( $C_{\rm quat}$ ), 140.6 ( $C_{\rm quat}$ ), 112.4 ( $C-H_{arom}$ ), 111.9 ( $C_{quat}$ ), 107.8 ( $NCH_2C=CH_2$ ), 102.4 ( $C-H_{arom}$ ), 65.2  $(OCH_2CH_2CH_2O)$ , 65.0  $(NCHCH_2OH)$ , 59.8  $(NCHCH_2OH)$ , 57.1  $(OCH_3)$ , 25 53.3  $(NCH_2C=CH_2)$ , 34.4  $(NCH_2C=CH_2CH_2)$ , 29.0  $(OCH_2CH_2CH_2O)$ ; MS (FAB) (Relative Intensity) 596 (M+·, 13), 484 (M-

NCH<sub>2</sub>CCH<sub>2</sub>CH<sub>2</sub>CHCH<sub>2</sub>OH, 14), 389 (10), 371 (29), 345 (5), 224 (8), 206 (44), 166 (100), 149 (24), 112 (39), 96 (34), 81 (28); IR (NUJOL<sup>®</sup>) 3600-3000 (br, OH), 3349 (NH<sub>2</sub>), 2922, 2852, 2363, 1615, 1591 (NH<sub>2</sub>), 1514, 1464, 1401, 1359, 1263, 1216, 1187, 1169, 1114, 1043, 891, 832, 761 cm<sup>-1</sup>.

(2S,4R) & (2S,4S) -1,1' - [[(Propane-1,3-diyl)dioxy]bis[(2-amino-5-methoxy-1,4-phenylene)carbonyl]]bis[2-(hydroxymethyl)-4-methylpyrrolidine] (77a).

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A solution of hydrazine (23 mg, 23  $\mu$ L, 0.72 mmol) in MeOH (5 mL) 10 was added dropwise to a solution of the diol 76 (95 mg, 0.145 mmol) and Raney Ni (20 mg) in MeOH (15 mL) heated at reflux. After 1 h at reflux TLC (90% CHCl3/MeOH) revealed some amine formation. The reaction mixture was treated with further Raney Ni (20 mg) and hydrazine (23 mg, 23 µL, 0.72 mmol) in MeOH (5 mL) 15 and was heated at reflux for an additional 30 min at which point TLC revealed complete reaction. The reaction mixture was then treated with enough Raney Ni to decompose any remaining hydrazine and heated at reflux for a further 1.5 h. Following cooling to room temperature the mixture was filtered through a sinter and 20 the resulting filtrate evaporated in vacuo. The resulting residue was then treated with  $CH_2Cl_2$  (30 mL), dried (MgSO<sub>4</sub>), filtered and evaporated in vacuo to provide the bis-amine 77a as a yellow oil (54 mg, 63%):  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>) (diastereoisomers)  $\delta$  6.73 (s,  $2H_{arom}$ ), 6.32 (s,  $2H_{arom}$ ), 4.60-4.30 25 (m, 2H, NCHCH<sub>2</sub>OH), 4.19 (t, 4H, J = 5.87 Hz, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 3.78-3.50 (m, 14H, NCHC $H_2$ OH, NC $H_2$ CHC $H_3$  and OC $H_3$ ), 2.40-1.55 (m, 8H,  $NCH_2CHCH_3$ ,  $OCH_2CH_2CH_2O$  and  $NCH_2CHCH_3CH_2$ ), 1.00-0.95 (m, 6H,

 $NCH_2CHCH_3$ ); MS (EI), m/z (relative intensity) 600 (M<sup>+</sup>·, 16), 459 (46), 345 (16), 206 (13), 186 (17), 180 (31), 166 (37), 149 (6), 142 (76), 100 (6), 98 (13), 97 (29), 84 (81), 69 (7), 55 (100).

(2S)-1,1'-[[(Propane-1,3-diyl)dioxy]bis[(2-allyloxycarbonylamino-5-methoxy-1,4-phenylene)carbonyl]]bis[2-(hydroxymethyl)-4-methylidenepyrrolidine] (78)

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Pyridine (0.47 mL, 0.46 g, 5.82 mmol) was added to a stirred solution of the bis-amine 77 (0.857 g, 1.44 mmol) in  $CH_2Cl_2$ (30 mL) at 0°C (ice/acetone). The cool mixture was then treated dropwise with a solution of allyl chloroformate (0.33 mL, 0.38 g, 10 3.15 mmol) in  $CH_2Cl_2$  (10 mL). After 2.5 h stirring at room temperature, the mixture was diluted with  $CH_2Cl_2$  (60 mL), washed with 1N HCl (2 X 50 mL),  $H_2O$  (80 mL), brine (80 mL), dried (MqSO<sub>4</sub>), filtered and evaporated in vacuo. The crude residue was purified by flash chromatography (70-100% EtOAc/Petroleum Ether) 15 to afford the allyl cartamate compound 78 as a slightly orange glass (0.548 g, 50%):  ${}^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  8.58 (br s, 2H, NH), 7.56 (s,  $2H_{arom}$ ), 6.78 (s,  $2H_{arom}$ ), 6.03-5.88 (m, 2H,  $NCO_2CH_2CH=CH_2$ ), 5.39-5.21 (m, 4H,  $NCO_2CH_2CH=CH_2$ ), 5.00 (br s, 2H,  $NCH_2C=CH_2$ ), 4.93 (br s, 2H,  $NCH_2C=CH_2$ ), 4.70-4.57 (m, 4H, 20  $NCO_2CH_2CH=CH_2$ ), 4.30-4.25 (m, 4H,  $OCH_2CH_2CH_2O$ ), 4.17-3.90 (m, 8H,  $NCHCH_2OH$  and  $NCH_2C=CH_2$ ), 3.81-3.54 (m, 8H,  $NCHCH_2OH$  and  $OCH_3$ ), 2.76 (dd, 2H, J = 8.52, 15.85 Hz,  $NCH_2C=CH_2CH_2$ ), 2.49-2.44 (m, 2H,  $NCH_2C=CH_2CH_2$ ), 2.36-2.28 (m, 2H,  $OCH_2CH_2CH_2O$ ); <sup>13</sup>C NMR (67.8) MHz, CDCl<sub>3</sub>) δ 170.3 (NC=O<sub>amide</sub>), 153.8 (NC=O<sub>carbamate</sub>), 150.5 25  $(C_{\text{quat}})$ , 144.8  $(C_{\text{quat}})$ , 143.1  $(C_{\text{quat}})$ , 132.5  $(NCO_2CH_2CH=CH_2)$ , 130.7  $(C_{\text{quat}})$ , 118.1  $(NCO_2CH_2CH=CH_2)$ , 116.8  $(C_{\text{quat}})$ , 110.9  $(C-H_{\text{arom}})$ ,

108.1 (NCH<sub>2</sub>C= $CH_2$ ), 106.9 ( $C-H_{arom}$ ), 65.7 (NCO<sub>2</sub> $CH_2$ CH= $CH_2$ ), 65.4 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 65.1 (NCH $CH_2$ OH), 59.8 (NCHCH<sub>2</sub>OH), 56.5 (O $CH_3$ ), 53.9 (N $CH_2$ C= $CH_2$ ), 34.2 (NCH<sub>2</sub>C= $CH_2$ CH<sub>2</sub>), 29.7 and 29.2 (OCH<sub>2</sub> $CH_2$ CH<sub>2</sub>O); MS (FAB) (Relative Intensity) 765 (M<sup>+</sup>· + 1, 10), 652 (M-NCH<sub>2</sub>CCH<sub>2</sub>CH<sub>2</sub>CHCH<sub>2</sub>OH, 32), 594 (4), 539 (2), 481 (51), 441 (31), 290 (3), 249 (13), 232 (38), 192 (83), 166 (49), 149 (32), 114 (100).

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1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11s,11as)-10-(allyloxycarbonyl)-11-hydroxy-7-methoxy-2-methylidene-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one] (79)

A solution of the bis-alloc compound 78 (150 mg, 0.196 mmol) in  $CH_2Cl_2/CH_3CN$  (12 mL, 3:1) was treated with 4 Å powdered molecular sieves (0.2 g) and NMO (70 mg, 0.598 mmol). After 15 min stirring at room temperature, TPAP (7 mg, 19.9 µmol) was added 15 and stirring continued for a further 2 h at which time TLC (95% CHCl3/MeOH) indicated formation, of the fully cyclised product along with the presumed semi-cyclised product 79a, and unreacted starting material 78 present in the reaction mixture. mixture was then treated with a further quantity of NMO (35 mg, 20 0.299 mmol) and TPAP (3.5 mg, 9.96  $\mu mol), and allowed to stir for$ a further 0.5 h when TLC revealed reaction completion. solvent was evaporated in vacuo and the black residue was subjected to flash chromatography (98%  $CHCl_3/MeOH$ ) to provide the pure protected carbinolamine 79 as a white solid (47 mg, 32%): 1H 25 NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.23 (s,  $2H_{arom}$ ), 6.74 (s,  $2H_{arom}$ ), 5.90-5.65 (m, 2H,  $NCO_2CH_2CH=CH_2$ ), 5.57 (d, 2H, J = 8.24 Hz, NCHCHOH),

5.26-5.07 (m, 8H, NCH<sub>2</sub>C=CH<sub>2</sub> and NCO<sub>2</sub>CH<sub>2</sub>CH=CH<sub>2</sub>), 4.67-4.10 (m, 14H,  $NCO_2CH_2CH=CH_2$ ,  $NCH_2C=CH_2$ ,  $OCH_2CH_2CH_2O$  and OH), 3.89 (s, 6H,  $OCH_3$ ), 3.63 (m, 2H, NCHCHOH), 2.91 (dd, 2H, J = 8.79, 15.76 Hz,  $NCH_2C=CH_2CH_2$ ), 2.68 (d, 2H, J=16.10 Hz,  $NCH_2C=CH_2CH_2$ ), 2.42-2.24 (m, 2H,  $OCH_2CH_2CH_2O$ ); <sup>13</sup>C NMR (67.8 MHz,  $CDCl_3$ )  $\delta$  166.7 5  $(NC=O_{amide})$ , 150.1  $(C_{quat})$ , 149.0  $(C_{quat})$ , 141.7  $(C_{quat})$ , 131.7  $(\text{NCO}_2\text{CH}_2\textit{C}\text{H}=\text{CH}_2)\,,\ 130.6\ (\textit{C}_{\text{quat}})\,,\ 128.9\ (\textit{C}_{\text{quat}})\,,\ 128.8\ (\textit{C}_{\text{quat}})\,,\ 118.3$  $(NCO_2CH_2CH=CH_2)$ , 114.7  $(C-H_{arom})$ , 110.7  $(C-H_{arom})$ , 109.8  $(NCH_2C=CH_2)$ , 85.9  $(NCH_2CHOH)$ , 66.9  $(NCO_2CH_2CH=CH_2)$ , 66.0  $(OCH_2CH_2CH_2O)$ , 59.7 (NCHCHOH), 56.1  $(OCH_3)$ , 50.7  $(NCH_2C=CH_2)$ , 35.0 10  $(NCH_2C=CH_2CH_2)$ , 29.7 and 29.1  $(OCH_2CH_2CH_2O)$ ; MS (FAB) (Relative Intensity) 743  $(M^+ - 17, 16)$ , 725 (17), 632 (13), 574 (8), 548 (13), 490 (10), 481 (9), 441 (7), 425 (6), 257 (12), 232 (20), 192 (46), 166 (52), 149 (100), 91 (59); IR (NUJOL®) 3234 (br, OH), 2923, 2853, 2361, 1707, 1604, 1515, 1464, 1410, 1377, 1302, 15 1267, 1205, 1163, 1120, 1045, 999, 955, 768, 722 cm<sup>-1</sup>.

1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11aS)-7-methoxy-2-methylidene-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one] (80, SJG-136)

A catalytic amount of tetrakis(triphenylphosphine)palladium (11 mg, 9.52 μmol) was added to a stirred solution of the bis-alloc-carbinolamine 79 (139 mg, 0.183 mmol), triphenylphosphine (4.8 mg, 18.3 μmol) and pyrrolidine (27 mg, 0.380 mmol) in CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>CN (13 mL, 10:3) at 0°C (ice/acetone) under a nitrogen atmosphere. The reaction mixture was allowed to warm to room temperature and the progress monitored by TLC (95% CHCl<sub>3</sub>/MeOH). After 2 h 15 min TLC revealed the reaction was complete,

proceeding via the presumed half-imine product 261, to give a TLC spot which fluoresced brightly under UV. The solvent was evaporated in vacuo and the resulting residue subjected to flash chromatography (98% CHCl3/MeOH) to give the bis-imine target molecule 80 (SJG-136) as a pale orange glass (78 mg, 77%) which 5 was repeatedly evaporated in vacuo with CHCl3 to provide the imine form:  $[\alpha]_{D}^{21} = +357.7$  ° (c = 0.07, CHCl<sub>3</sub>); Reverse Phase HPLC ( $C_4$  stationary phase, 65% MeOH/ $H_2O$  mobile phase, 254 nm), Retention time = 6.27 min, % Peak area = 97.5%; <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) (imine form)  $\delta$  7.68 (d, 2H, J = 4.4 Hz, HC=N), 7.49 (s, 10  $2H_{arom}$ ), 6.85 (s,  $2H_{arom}$ ), 5.20 (s, 2H,  $NCH_2C=CH_2$ ), 5.17 (s, 2H,  $NCH_2C=CH_2$ ), 4.46-4.19 (m, 4H,  $OCH_2CH_2CH_2O$ ), 3.92 (s, 6H,  $OCH_3$ ), 3.89-3.68 (m, 6H,  $NCH_2C=CH_2$  and NCHHC=N), 3.12 (dd, 2H, J=8.61, 16.21 Hz,  $NCH_2C=CH_2CH_2$ ), 2.68 (d, 2H, J=16.30 Hz,  $NCH_2C=CH_2CH_2$ ), 2.45-2.38 (m, 2H, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O);  $^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>) (imine 15 form)  $\delta$  164.7 (NC=0), 162.6 (HC=N), 150.7 ( $C_{quat}$ ), 147.9 ( $C_{quat}$ ), 141.5  $(C_{\text{quat}})$ , 140.6  $(C_{\text{quat}})$ , 119.8  $(C_{\text{quat}})$ , 111.5  $(C_{\text{Harom}})$ , 110.7  $(C-H_{arom})$ , 109.4  $(NCH_2C=CH_2)$ , 65.4  $(OCH_2CH_2CH_2O)$ , 56.1  $(OCH_3)$ , 53.8 (NCHHC=N), 51.4  $(NCH_2C=CH_2)$ , 35.4  $(NCH_2C=CH_2CH_2)$ , 28.8 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O); MS (FAB) (Relative Intensity) (imine form) 773 (M<sup>+</sup>· 20 + 1 + (Thioglycerol adduct X 2), 3), 665 (M+· + 1 + Thioglycerol adduct, 7), 557  $(M^{+} + 1, 9)$ , 464 (3), 279 (12), 257 (5), 201 (5), 185 (43), 166 (6), 149 (12), 93 (100); IR (NUJOL®) 3600-3100 (br, OH of carbinolamine form), 2923, 2849, 1599, 1511, 1458, 1435, 1391, 1277, 1228, 1054, 1011, 870, 804, 761, 739 cm<sup>-1</sup>. 25

Example 2(e): Synthesis of PBD with ketone on C-ring (172, UP-2067) (see Figure 10)

(2S) (4R) -N-[4-benzyloxy-5-methoxy-2-(2', 2', 2'-

trichloroethoxy) carbonyl]-2-(tert-butyldimethylsilyloxymethyl)-4
5 hydroxypyrrolidine (168)

A solution of 2,2,2-trichloroethylchloroformate (8.74 g, 5.68 mL, 41.2 mmol) in dichloromethane (50 mL) was added to a solution of 4 (18.2g, 37.5 mmol) and pyridine (5.92 g, 6.1 mL, 75.0 mmol) in dry dichloromethane (200 mL) at 0°C under a nitrogen atmosphere. The reaction mixture was allowed to stir overnight at room temperature and was then washed with saturated copper sulphate solution (100 mL), water (100 mL) and brine (100 mL). The organic phase was dried over magnesium sulphate, filtered and

product 168 (22.01 g, 33.2 mmol, 89%) which was used in the subsequent reaction without further purification.  $^{1}H$  NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  9.31 (bs, 1H); 7.48 (s, 1H); 7.45-7.28 (m, 5H); 6.82 (s, 1H); 5.17 (bs, 2H); 4.89 (d, J = 11.9 Hz, 1H); 4.70 (d,

excess solvent removed by rotary evaporation to afford the

J = 11.9 Hz, 1H); 4.56 (bs, 1H); 4.40 (bs, 1H); 4.20-4.00 (m,  $20 \quad 1\text{H}); 3.95-3.40 \text{ (m, 7H)}; 2.40-2.00 \text{ (m, 2H)}; 0.09 \text{ (s,9H)}; 0.04 \text{ (s,}$   $6\text{H}). \quad ^{13}\text{C NMR} \text{ (67.8 MHz, CDCl}_3) \delta 169.2, 152.1, 150.2, 136.1,$  128.6, 128.1, 127.7, 111.6, 106.2, 95.2, 74.4, 70.7, 70.5, 62.1,

57.2, 56.4, 35.4, 25.8, 18.1, -5.46.

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(2S)-N-[4-benzyloxy-5-methoxy-2-(2', 2', 2'-

25 trichloroethoxy) carbonyl amino] -2-(tert-

butyldimethylsilyloxymethyl) -4-oxopyrrolidine (169)

A solution of DMSO (7.80 g, 99.8 mmol) in dry dichloromethane

(18 mL) was added dropwise, over 30 minutes, to a solution of oxalyl chloride (6.34 g, 49.9 mmol) in dry dichloromethane (25 mL) at - 45°C under a nitrogen atmosphere and the reaction mixture allowed to stir for a further 15 minutes. A solution of the substrate 168 (22.01 g, 33.3 mmol) in dichloromethane 5 (50 mL) was added dropwise over 40 minutes to the reaction mixture, which was then allowed to stir for 45 minutes at - 45°C. Finally, neat triethylamine (23.52 g, 232.9 mmol) was added dropwise over 30 minutes and the reaction mixture allowed to stir at  $-45^{\circ}\text{C}$  for 15 minutes. The reaction mixture was allowed to 10 warm to room temperature, diluted with water (150 mL) and the organic phase washed with dilute HCl (1N, 100 mL), water (100 mL) and brine (100 mL). The organic phase was dried over magnesium sulphate, filtered and concentrated in vacuo to afford the crude product which was subjected to column chromatography 15 (ethyl acetate/40-60 petroleum ether, 50:50). Removal of excess eluent afforded the product (20.15 g, 92% yield). <sup>1</sup>H NMR (270 MHz, CDCl3) δ 7.88 (bs, 1H); 7.49-7.28 (m, 5H); 6.80 (s, 1H); 5.22 (d, J = 12.1 Hz, IH); 5.17 (d, J = 12.1 Hz, IH); 4.80 (bs, 2H); 4.10-3.60 (m, 8H); 2.75 (dd, J = 18.0, 9.5 Hz, 1H); 2.52 (d, 20 J = 18.0 Hz, 1H; 0.87 (s, 9H); 0.06 (s, 3H); 0.05 (s, 3H).  $^{13}\text{C}$ NMR (67.8 MHz) & 208.7, 168.8, 151.8, 150.6, 144.7, 136.0, 128.5, 128.1, 127.7, 110.9, 106.4, 95.2, 74.4, 70.7, 66.0, 56.8, 56.4, 39.4, 25.8, 18.0, -5.7.

(2S)-N-[4-benzyloxy-5-methoxy-2-(2', 2', 2'trichloroethoxy)carbonyl amino]-2-(hydroxymethyl)-4oxopyrrolidine (170)

Glacial acetic acid (60 mL) and water (20 mL) were added to a solution of ketone 169 (9.44 g, 14.3 mmol) in THF (20 mL) and the 5 reaction mixture allowed to stir for 3 hr. (reaction complete by TLC). The reaction mixture was diluted with dichloromethane (200 mL) and neutralized dropwise with sat. sodium bicarbonate (1.5 L) in a 5 L flask (effervescence!). The phases were allowed to separate and the aqueous layer extracted with dichloromethane 10 (2 x 100 mL). The combined organic layers were washed with brine and dried over magnesium sulphate. Removal of excess solvent afforded the crude product which was subjected to column chromatography on silica (ethyl acetate/40-60 petroleum ether, 50:50) to give the pure product (6.44 g, 83%). <sup>1</sup>H NMR (270 MHz,15 CDCl<sub>2</sub>)  $\delta$  8.77 (bs, 1H); 7.57 (s, 1H); 7.46-7.28 (m, 5H); 6.83 (s,1H); 5.13 (s, 2H); 4.85-4.70 (m, 3H); 4.07-3.60 (m, 7H); 2.77 (dd, J = 18.5, 9.5 Hz, 1H); 2.54 (d, J = 18.5Hz, 1H). <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>) δ 209.0, 169.4, 152.3, 150.6, 145.5, 136.0, 130.0, 128.6, 128.3, 127.6, 110.9, 107.4, 95.2, 74.5, 70.8, 64.4, 20 60.4, 56.6, 55.9, 39.5.

(11s, 11aS)-4-benzyloxy-11-hydroxy-5-methoxy-4-oxo-10-(2', 2', 2'-trichloroethoxy)carbonyl-amino 1, 10, 11, 11a-tetrahydro-5H-pyrrolo-[2,1-c][1,4]benzodiazepin-5-one (171)

A solution of DMSO (4.45 g, 4.04 mL, 56.9 mmol) in dry dichloromethane (25 mL) was added dropwise, over 5 minutes, to a solution of oxalyl chloride (3.58 g, 49.9 mmol) in dry

dichloromethane (14 mL) at -60°C under a nitrogen atmosphere and the reaction mixture allowed to stir for a further 15 minutes. A solution of the substrate 170 (10.93 g, 20.0 mmol) in dichloromethane (25 mL) was added dropwise over 30 minutes to the reaction mixture, which was then allowed to stir for 30 minutes 5 at -60°C. Finally, neat triethylamine (11.15 g, 232.9 mmol) was added dropwise over 30 minutes and the reaction mixture allowed to stir at -60°C for 15 minutes. The reaction mixture was allowed to warm to room temperature, diluted with water (150 mL) and the organic phase washed with dilute HCl (1N, 100 mL), water 10 (100 mL) and brine (100 mL). The organic phase was dried over magnesium sulphate, filtered and concentrated in vacuo to afford the crude product which was subjected to column chromatography (ethyl acetate/40-60 petroleum ether, 50:50). Removal of excess eluent afforded the product 171 (9.66 g, 89 % yield). 15 (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.45-7.33 (m, 5H); 7.27 (s, 1H); 6.95 (s, 1H); 5.76 (d, J = 9.9 Hz, 1H); 5.52-5.00 (m, 3H), 4.33 (d, J = 6.8Hz, 1H); 4.30 (d, J = 19.2 Hz, 1H); 4.00-3.70 (m, 5H); 2.98 (dd, J = 20.0, 10.4 Hz, 1H); 2.94 (d, J = 20.0 Hz, 1H). <sup>13</sup>C NMR (67.8 MHz) δ 207.7, 167.5, 154.5, 152.6, 150.8, 149.6, 135.8, 20 128.9-127.3, 124.0, 114.5, 110.8, 95.0, 86.6, 75.0, 71.1, 56.8, 56.2, 52.6, 40.2.

(11aS)-4-benzyloxy-5-methoxy-4-oxo-1,10,11,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (172)

Cadmium/lead couple (1.15 g) was added to a solution of cyclized ketone (1 g, 1.84 mmol) in THF (5 mL) and aqueous ammonium acetate (1N, 15 mL). The reaction mixture was allowed to stir

for 90 minutes and then filtered through celite. The celite pad was washed with ethyl acetate (2 x 25 mL) and the organic layer separated. The organic layer was washed with brine (50 mL) and dried over magnesium sulphate.—Removal of excess solvent

5 followed by column chromatography afforded the pyrrolobenzodiazepine 172 (0.324 g, 0.93 mmol). <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) δ 7.75 (d, J = 4.4 Hz, 1H); 7.51 (s, 1H); 7.46-7.27 (m, 5H); 5.23 (d, J = 12.3 Hz, 1H); 5.17 (d, J = 12.3 Hz, 1H), 4.24-4.40 (m, 3H), 3.96 (s, 3H), 3.12 (dd, J = 19.6, 8.8 Hz, 1H); 2.99 (dd, J = 5.0 Hz, 1H). <sup>13</sup>C NMR (67.8 MHz) δ 206.7, 165.5, 161.4, 151.1, 148.5, 140.5, 136.0, 128.7-127.1, 118.9, 111.7, 111.3, 70.9, 56.4, 53.4, 51.0, 40.0.

# Example 3: Synthesis of Compounds of formula III Overview of Synthesis

The Biaryl PBDs 136, 138 and 140 were obtained by removal of the 15 Troc protecting group from the protected carbinolamines 135, 137 and 139. For compounds 136 and 138 the deprotection method of Dong et al, was employed (Cd/Pb, ammonium acetate buffer), however, this approach could not be applied to the preparation of 140 as this molecule contained a nitro group sensitive to the 20 Cd/Pb couple. In this case a novel deprotection procedure involving the use of tetrabutyl ammonium fluoride was used. The protected biaryl carbinolamines were prepared by the Suzuki reaction, the common 7-iodo substituted protected carbinolamine 134 was exposed to the appropriate boronic acid in the presence 25 of a palladium catalyst, this reaction is of wide scope as over 70 boronic acids are commercially available. The iodo

substituted protected carbinolamine 134 was furnished by Swern oxidation of the primary alcohol 133. The Swern procedure was particularly effective in this case but other oxidizing agents such as the -Dess-Martin reagent, TPAP or pyridine sulphur trioxide complex and DMSO could also be employed. The primary alcohol 133 was afforded by coupling commercially available pyrrolidinemethanol to the Troc protected anthranilic acid chloride obtained by 132 by treatment with oxalyl chloride. The Troc protected acid was in turn prepared by exposing the anthranilic acid 131 to 2,2,2-trichloroethyl chloroformate. Other protecting groups can be used in place of Troc such as Nvoc, Teoc and Fmoc but care must be taken in choosing a protecting group as some groups such as Boc spontaneously form the isatoic anhydride when exposed to oxalyl chloride prior to the coupling step.

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The 9-methoxy PBD (101) was prepared in an analogous fashion demonstrating the versatility of the approach.

The 8-amino PBD (151) was prepared by the removal of a Troc protecting group from the amino substituted protected carbinolamine 150. The free amine was obtained by removal of an Fmoc protecting group under standard conditions (piperidine/DMF) from the protected carbinolamine 149. Swern oxidation of the primary alcohol 148 furnished 149 in good yield, the substrate for oxidation reaction was prepared by Fmoc protection of the aniline 147. Reduction of the nitro compound 146, with tin chloride furnished the aniline, hydrogenation could not be

employed to reduce the nitro group as the Troc system does not withstand these conditions. The nitro compound 146 was prepared by the coupling of the acid chloride derived from 145 with pyrrolidinemethanol in the presence of base. Finally, the protected anthranilic acid 145 was furnished by exposing the commercially available 4 nitro anthranilic acid 144 to Troc Chloroformate.

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The 8-benzyloxy-7,9-dimethoxy PBD (143, UP2022) was prepared by a slightly different approach which does not involve the use of anthranilic acid starting materials but proceeds through 2nitrobenzoic acid intermediates. The PBD was obtained from the protected carbinolamine 142 by removal of the Troc protecting group under the usual conditions. The protected carbinolamine was furnished by Swern oxidation of primary alcohol 141 which in turn was prepared by selective protection of the amino alcohol 126 as the Troc carbamate by exposure to Troc Chloroformate in the presence of pyridine. The amino alcohol was obtained by reduction of the nitro compound 125 with Raney Nickel and hydrazine (again hydrogenation could not be employed due to the presence of a benzyl group). The nitro alcohol 125 was prepared by coupling pyrrolidine methanol to the requisite 2-nitrobenzoic acid 124. This nitro benzoic acid was not commercially available and was prepared in four steps from the available syringic acid Nitration of the ester 122 was proceeded smoothly using Copper nitrate in acetic anhydride, the ester 122 was obtained by standard methods.

The PBDs 96, 113 and 120 were obtained in an identical fashion from the 2-nitrobenzoic acids 19, 108 and 115.

The dimer 90 was prepared in an analogous fashion from the core nitro compound 85; the core was assembled by joining together two units of the phenol 84 via Mitsonobu etherification. The phenol 84 was derived from syringic acid 83 in a three step synthesis, the crucial step being the nitration of 82 which was performed with 70% nitric acid.

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The phenolic PBD 130 was prepared by an analogous route to that used for the synthesis of the PBD 143, however the requirement to incorporate a phenolic group prompted the use of a different protecting group, Teoc. The free PBD was obtained by treating the Teoc protected carbinolamine 129 with TBAF in warm acetonitrile. The phenol 129 was unmasked by the hydrogenolysis of the benzyloxy moiety of 128 in the presence of the Teoc protecting group (Troc would not survive under these conditions). The benzyloxy compound 128 was obtained by Swern oxidation of the primary alcohol 127 which was prepared by treating the amino alcohol 126 with Teoc chloroformate in the presence of base.

# Example 3(a): Synthesis of the C9/C9'-Dimethoxy PBD Dimer (90, DRH-165) (see Figure 11)

#### O-Acetylsyringic acid (82)

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A suspension of syringic acid 81 (10.0 g, 50.5 mmol) in acetic anhydride (30.0g, 27.7 mL, 294.1 mmol) was warmed gently until a clear solution was obtained. Fused sodium acetate (0.5g, 6.10 mmol) was added to the solution which was allowed to stir for 16 h at room temperature. The solution was poured into water (100 mL) and stirred thoroughly to ensure hydrolysis of any excess anhydride. Crude O-Acetyl-syringic acid was recrystallized from water to afford the product as an off-white powder (11.2 g, 46.7 mmol). H¹ NMR (270 MHz, CDCl<sub>3</sub>) δ 7.36 (s, 2H), 5.94 (br s, 1H), 3.87 (s, 6H), 2.35 (s, 3H). HRMS calcd for 240.0634, found 240.0637

#### 4-Acetoxy-3,5-dimethoxy-2-nitrobenzoic acid (83)

Fuming nitric acid (5.2 mL) was added, carefully, to a solution of o-acetylsyringic acid 82 (11.1 g, 46.2 mmol) in acetic anhydride (33 g, mmol) at 5°C and the reaction mixture was then allowed to stir for 3 h at room temperature. The reaction mixture was poured over ice (300 mL) and the yellow precipitate was collected by filtration, washed with water (3 x 100 mL) and dried in vacuo to afford the product as a pale yellow solid (12.4 g). H¹ NMR (270 MHz, CDCl₃) δ 7.37 (s, 1H), 3.92 (s, 3H), 3.90 (s, 3H), 2.39 (s, 3H).

Methyl 3,5-dimethoxy-4-hydroxy-2-nitrobenzoate (84)

A catalytic amount of DMF (5 drops) was added to a solution of oxalyl chloride (6.3 g, 49.8 mmol) and o-nitrobenzoic acid 83 (12.4 g, 45.2 mmol) in anhydrous THF (100 mL) and the reaction mixture allowed to stir at room temperature for 16 h. 5 resulting acid chloride was quenched dropwise with anhydrous methanol (100 mL) at 0 °C. The reaction mixture was treated with potassium carbonate and allowed to stir at room temperature for 3 Excess solvent was removed by rotary evaporation at reduced h. pressure and the residue dissolved in water. The aqueous 10 solution was acidified to pH 8 and the resulting white precipitate was collected by filtration, washed with water (2 x 100 mL) and dried to afford the product as an off-white solid (10.6 q, 83%).  $H^1$  NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  10.07 (br s, 1H), 7.26 (s, 1H), 3.97 (s, 3H), 3.91 (s, 3H), 3.85 (s, 3H). 15

1', 3'-Bis(4-carboxy-2,6-dimethoxy-5-nitrophenoxy)propane (85) Diethylazidodicarboxylate (7.19 g, 41.3 mmol) was added dropwise over 0.5 h to a cooled, stirred solution of the phenol 84 (10.61 g, 41.3 mmol) and TPP (16.24 g, 61.9 mmol) in anhydrous THF (100 mL), and allowed to stir for 1 h. A solution of 1,3-propanediol (1.57q, 20.6 mmol) in THF (30 mL) was added dropwise and the reaction mixture allowed to stir for 16 h. The reaction mixture was then treated with 1N aqueous NaOH (200 mL) and heated at reflux for 3 h. Excess solvent was removed by rotary evaporation 25 under reduced pressure to afford an aqueous suspension which was extracted with EtOAc (3 x 300 mL). The aqueous extract was acidified with concentrated HCl and the precipitate collected by

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vacuum filtration. The precipitate was suspended in water (500 mL) and after stirring for 10 minutes, the suspension was filtered to afford the product as an orange solid (6.11 g, 60%). H<sup>1</sup> NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.32 (s, 2H), 4.36 (t, 4H,), 3.92 (s, 6H), 3.90 (s, 6H), 2.20 (t, 2H).

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(2S)-1,1'-[[(propane-1,3-diyl)dioxy]bis[2-nitro-3,5-dimethoxy-1,4-phenylene)carbonyl]]bis[2-(hydroxymethylpyrrolidine] (86) A catalytic amount of DMF (3 drops) was added to a solution of the acid 85 (6.1g, 12.4 mmol) and oxalyl chloride (2.37 mL, 3.45 g, 27.2 mmol) in anhydrous DCM (60 mL) and the reaction mixture allowed to stir at room temperature for 16 h. The resulting acid chloride was added dropwise over 0.5 h to a stirred solution of TEA (6.26 g, 61.8 mmol) and pyrrolidinemethanol (2.75 g, 27.2 mmol) in anhydrous DCM (60 mL) at -10°C. The reaction mixture was then allowed to stir at room temperature for 6 h. The reaction mixture was washed with 1N HCl (3 x 100 mL), water (3 x 100 mL), saturated NaHCO<sub>3</sub> (3 x 100 mL), brine (3 x 100 mL) and dried over MgSO4. Removal of excess solvent by rotary evaporation under reduced pressure afforded the product as a yellow glass (8.25 g, 11.9 mmol).  $H^1$  NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  6.66 (s, 2H), 4.32-4.26 (m, 6H), 3.98 (s, 6H), 3.90 (s, 6H), 3.86-3.67 (m, 4H), 3.41-3.27 (m, 4H), 2.23-2.12 (m, 2H), 2.11-1.72 (m, 8H).

(2S)-1,1'-[[(propane-1,3-diyl)dioxy]bis[2-amino-3,5-dimethoxy-1,4-phenylene)carbonyl]]bis[2-(hydroxymethylpyrrolidine] (87) Hydrazine (3.45 g, 107.9 mmol) was added dropwise to a solution of 86 (1g, 1.45 mmol) in anhydrous methanol (40 mL) heated at reflux over Raney nickel (5 g, slurry). Heating was continued 5 for a further 3 h after which time the reaction mixture was allowed to cool and filtered through celite to remove excess Raney nickel. The filtrate was evaporated to dryness and dissolved in DCM (200 mL) and the organic solution washed with water (2 x 100 mL), brine (2 x 100 mL) and dried over  $MgSO_4$ . 10 Filtration and evaporation of excess solvent in vacuo afforded the product as a pink glass (5.59 g, 8.9 mmol, 98%). H<sup>1</sup> NMR (270 mmol, 98%)MHz, CDCl<sub>3</sub>)  $\delta$  6.54 (s, 2H), 4.35 (br s, 2H), 4.29 (t, 4H), 3.85 (s, 3H), 3.83-3.46 (m, 14H), 2.20-2.13 (m, 2H), 1.97-1.66 (m, 15 8H).

(2s)-1,1'-[[(propane-1,3-diyl)dioxy]bis[2-(2',2',2'trichloroethoxycarbonyl)amino-3,5-methoxy-1,4phenylene)carbonyl]]bis[2-(hydroxymethylpyrrolidine] (88)
A solution of 2,2,2-trichloroethylchloroformate (1.45 g, 6.86)

20 mmol, 1.9 eq) in dry DCM (10 mL) was added dropwise over the space of 0.5 h to a solution of 87 (2.28 g, 3.6 mmol) and pyridine (1.14 g, 14.4 mmol, 4 eq) in dry DCM (50 mL) and allowed to stir for 16 h at room temperature. The reaction mixture was diluted with DCM (200 mL) and washed with 1N HCl (3 x 200 mL),

25 H<sub>2</sub>O (3 x 200 mL), brine (2 x 300 mL) and dried over anhydrous MgSO<sub>4</sub>. Purification by flash chromatography (silica gel, EtoAc) afforded the product as a pale yellow glass (1.43 g). H<sup>1</sup> NMR (270

MHz, CDCl<sub>3</sub>) Rotamers  $\delta$  9.21 and 8.40 (2 x br s, 2H), 6.49 and 6.54 (2 x s, 2H), 5.08-3.59 (m, 26H), 3.33-3.30 (m, 4H), 2.04-1.69 (m, 10H).

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1,1'-[[Propane-1,3-diyl)dioxy]bis[(11S,11aS)-10-(2',2',2'-trichloroethoxycarbonyl)-11-hydroxy-7,9-dimethoxy-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one. (89)

A solution of dry DMSO (14.9 mmol, 1.17g, 1.06 mL) in dry DCM (5 mL) was added dropwise over 20 minutes to a stirred solution of oxalyl chloride in DCM (7.38 mmol, 3.69 mL of a 2N solution in DCM) under a nitrogen atmosphere at -45° C. After stirring for an additional 15 minutes, a solution of 88 (2.58 g, 2.63 mmol) in dry DCM (5m L) was added dropwise over 45 minutes at -45° C and stirred for 45 minutes at -45° C. TEA (2.12 g, 21.0 mmol) was added dropwise over 30 minutes and stirred for a further 15 minutes. The reaction mixture was allowed to warm to room temperature, and diluted with water (100 mL). The organic layer was washed with 1N HCl (3 x 100 mL), water (3 x 100 mL), brine (3 x 100 mL) and dried over anhydrous MgSO4. Filtration and evaporation of the solvent in vacuo afforded the product as a yellow glass (0.73 g).  $H^1$  NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.06 (s, 2H), 5.61 (dd, 2H, J = 3.39, 9.9 Hz), 4.74 (d, 2H, J = 11.72 Hz), 4.62 (d, 2H, J = 11.91 Hz), 4.29-4.21 (m, 6H), 3.97-3.46 (m, 16H),2.28-2.01 (m, 10H).

## Preparation of 10% Cd/Pb couple

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Yellow lead oxide (litharge, 1.8 g, 4.9 mmol) was dissolved in warm 50% aq. AcOH (50 mL) and the sclution was slowly added to a vigorously stirred suspension of Cd dust (Aldrich, 100 mesh, 5.46 g, 49 mmol) in deionised water (100 mL). The Cd darkened as Pb deposited on its surface, and formed clumps that were gently broken up with a glass rod. The dark non-pyrophoric Cd/Pb couple was filtered, washed with water, acetone, crushed and dried prior to storage and use.

1,1'-[[Propane-1,3-diyl)dioxy]bis[(11aS)-7,9-dimethoxy-1,2,3,11a-10 tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one. (90) Cadmium/lead couple (3.8 mmol Cd, 0.47 g of Cd\Pb couple) was added to a vigorously stirred solution of 89 (0.76 g, 0.8 mmol) in THF (10 mL) and 1N NH4OAc (10 mL) and stirring continued for 2.5 h. The reaction mixture was diluted with DCM (150 mL) and 15 dried over MgSO<sub>4</sub>. Filtration and evaporation of the solvent in vacuo afforded the product as a yellow glass (0.32 g, 0.55 mmol, 71%).  $H^1$  NMR (270 MHz, CDCl<sub>3</sub>) mixture of C11/C11'R/S carbinolamines  $\delta$  7.08 (s, 2H), 5.53 (br s, 2H), 5.38 (br s, 2H), 4.90 (d, 2H, J = 9 Hz), 4.79 (d, 2H, J = 9 Hz), 4.38-3.54 (m, 20 22H), 2.27-1.79 (m, 10H). MS (FAB) m/e (relative intensity) 594 (M+2, 27%), 593 (M+1, 69%)

# Example 3(b): Synthesis of the C7-Methoxy PBD (96, DRH-271) (see Figure 12)

## N-(3-Methoxy-2-nitrobenzoyl)pyrrolidin-2-methanol (92)

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A catalytic amount of DMF (2 drops) was added to a stirred solution of 3-methoxy-2-nitro-benzoic acid 91 (5.01 g, 25.4 mmol) and oxalyl chloride (3.54 g, 27.9 mmol) in dry CHCl<sub>2</sub> (50 mL) under a nitrogen atmosphere. The reaction mixture was allowed to stir overnight, before being used directly in the preparation of 92. A solution of the acid chloride in anhydrous CHCl<sub>2</sub> (50 mL) was added dropwise over 1 h to a vigorously stirred solution of pyrrolidinemethanol (2.57 g, 25.4 mmol) and TEA (6.42 g, 63.6 mmol) in anhydrous CHCl<sub>2</sub> (50 mL) under a nitrogen atmosphere at 0°C and allowed to stir overnight at room temperature. The reaction mixture was washed with 1N HCl (1 x 100 mL), H<sub>2</sub>O (3 x 100 mL) and brine (3 x 100 mL). The organic layer was dried over anhydrous MgSO<sub>4</sub>, and evaporation of the solvent afforded a brown oil (6.37 g, 22.7 mmol, 89%).

#### N-(2-Amino-3-Methoxybenzoyl)pyrrolidin-2-methanol (93)

Hydrazine hydrate (4.37 g, 136.4 mmol) was added dropwise to a

20 solution of 92 (6.37 g, 22.7 mmol) in gently refluxing methanol

(100 mL) over Raney nickel (2.4 g, slurry). The resulting

vigorous evolution of hydrogen gas subsided after approximately

10 mins and the reaction was deemed to be complete by TLC after 2

h. The reaction mixture was filtered through celite and the solvent evaporated. Distilled water (100 mL) was added to the residue, and the aqueous mixture was extracted with EtOAc (3 x 100 mL) and washed with  $\rm H_2O$  (3 x 100 mL) and brine (3 x 100 mL) and dried over anhydrous MgSO<sub>4</sub>. Evaporation of the solvent afforded a brown glass (5.49 g, 21.8 mmol) as a single spot by TLC.

## N-(3-Methoxy-2-((2',2',2'-

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trichloroethoxy) carbonylaminobenzoyl) pyrrolidin-2-methanol (94) 10 A solution of 2,2,2-trichloroethyl chloroformate (4.61 g, 21.8 mmol) in distilled dichloromethane (50 mL) was added dropwise over 0.5 h to a stirred solution of the substrate, 93 (5.46 q, 21.8 mmol) and anhydrous pyridine (3.44 g, 43.5 mmol) in distilled dichloromethane (100 mL) at 0°C. The reaction mixture 15 was allowed to stir for 2.5 hours at which time TLC showed reaction to be complete. The reaction mixture was diluted with anhydrous DCM (100 mL) and washed with 1N HCl (2 x 200 mL),  $H_2O$ (200 mL), brine (200 mL) and dried over anhydrous MgSO4. Evaporation of the solvent afforded a brown oil which was 20 purified by flash column chromatography eluting with EtOAc to afford the product as a yellow solid (6.14 g, 14.4 mmol);  $^{1}\mathrm{H}$  NMR  $(270 \text{ MHz}, \text{CDCl}_3) \delta 1.75-2.25 \text{ (m, 4H)}, 3.4-3.75 \text{ (m, 2H)}, 3.8 \text{ (s, }$ 3H), 3.85-4.2 (m, 2H), 4.40 (m, 1H), 4.73-4.86 (m, 2H), 6.86-6.97 (m, 2H), 7.85 (br d, 1H, J = 9Hz); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$ 25 169.9, 155.6, 152.4, 128.2, 127.8, 123.6, 116.0, 113.0, 95.4, 74.4, 65.9, 60.9, 55.7, 51.0, 28.3, 24.9.

(11*S*, 11a*S*)-10-(2',2',2'-trichloroethoxy) carbonyl-7-methoxy-11-hydroxy-1,2,3,10,11,-11a-hexahydro-5*H*-pyrrolo[2,1-c][1,4]benzodiazepin-5-one.(95)

Anhydrous DMSO (3.14 g, 40.2 mmol) in dry DCM (25 mL) was added dropwise over 5 mins to a stirred solution of oxalyl chloride 5 (2.53 q, 9.96 mL of a 2 N solution in DCM) under a nitrogen atmosphere at -50°C. After stirring for 5 minutes, the substrate 94 (6.03 q, 14.2 mmol) in dry DCM (25 mL) was added dropwise over 45 mins to the reaction mixture, which was then allowed to stir for a further 45 mins at -50°C after the addition of the 10 substrate. Dry TEA (5.72 g, 56.64 mmol) was added dropwise to the mixture over 0.5 h and the reaction mixture allowed to stir for a further 15 minutes. The reaction mixture was left to warm to room temperature and diluted with  ${\rm H_2O}$  (100 mL). The organic phase was washed with 1N HCl (2 x 200 mL),  $H_2O$  (2 x 200 mL), 15 brine (2 x 200 mL) and dried over anhydrous MgSO4. The solvent was evaporated to afford a yellow oil (6.68 g). The oil was subjected to flash chromatography with EtOAc as eluent to afford the product as a yellow solid (5.87 g, 13.9 mmol); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  1.99-2.14 (m, 4H), 3.45-3.77 (m, 2H), 3.85 (s, 3H), 4.19 (br s, 1H), 4.28 (d, 1H, J = 11.91 Hz), 5.14 (d, 1H, J =11.91 Hz), 5.66 (d, 1H, J = 9.71 Hz), 6.97-7.02 (m, 1H), 7.23-7.27 (m, 2H); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>) d 166.8, 159.1, 154.7, 134.3, 131.5, 129.9, 126.6, 118.106, 112.5, 112.3, 95.0, 86.0, 75.2, 75.1, 59.8, 55.7, 46.7, 46.4, 28.7, 23.0, 21.0, 14.2. 25

7-methoxy-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-

c] [1,4]benzodiazepin-5-one (96)

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10% Cd/Pb couple (2.50 g, 20 mmol Cd) was added to a rapidly stirring solution of 95 (1.71 g, 4.03 mmol) in a mixture of THF (30 mL) and 1N NH40Ac (30 mL). Upon addition, the solution turned cloudy and after 2 h TLC showed the reaction to be complete. The reaction mixture was diluted with EtOAc (150 mL) and dried over anhydrous MgSO4. The solids were filtered and rinsed with EtOAc (50 mL). Removal of excess solvent by rotary evaporation under reduced pressure afforded the product as a yellow solid (0.84 g, 3.6 mmol, 90%)

# Example 3(c): Synthesis of the C7-Methoxy PBD (101, AG/140) (see Figure 13)

3-methoxy-2-(2',2',2'-trichloroethoxycarbonylamino)benzoic acid

(98)

2-amino-3-methoxybenzoic acid 97 (1 g, 6.0 mmol) and pyridine

(0.97 mL, 12.0 mmol) were dissolved in dry dichloromethane (30 mL). The resulting mixture was cooled and Troc-Cl (0.9 mL, 6.6 mmol) was added drop wise. The reaction mixture was allowed to

20 stir overnight at room temperature, then washed with HCl (1N, 50 mL), water (50 mL) and brine (50 mL). The organic phase was dried over MgSO<sub>4</sub> and evaporated to yield 1.42 g of crude product, which was used in the next step without further purification.

N-(3-methoxy-2-(2',2',2'-trichloroethoxycarbonylamino)benzoyl)pyrrolidine-2-methanol (99)

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Oxalyl chloride (0.57 mL, 6.58 mmol) together with 2 drops of dry DMF was added to a solution of the crude product obtained from the previous reaction in dry dichloromethane (20 mL). After initial strong effervescence, the mixture was allowed to stir at room temperature overnight. The resulting acid chloride was added drop wise, over 30 minutes to a solution of 2S-(+)pyrrolidinemethanol (0.66 g, 6.58 mmol) and TEA (2.1 mL, 14.95 mmol) in dry dichloromethane (20 mL) at -16°C. Once coupling was complete the reaction mixture was diluted with ethyl acetate (20 mL), and washed with 1N HCl (2 x 25 mL), satd. aqueous NaHCO<sub>3</sub> (2 x 25 mL), water (25 mL) and brine (25 mL). The organic layer was then dried over MgSO<sub>4</sub> and evaporated to give a yellow oil. The crude product was purified by flash chromatography (petroleum ether/ethyl acetate, 50/50) to afford 0.54 g, of a pale yellow oil: <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) d 1.6 - 1.8 (m, 1H); 1.81 - 2.0 (m, 2H); 2.02 - 2.21 (m, 1H); 3.4 (m, 1H); 3.6 (m, 2H); 3.86 (m, 4H); 4.22 (dd, J = 5.1, J = 12.3 Hz, 1H); 4.72 (d, J = 12 Hz, 1H);4.79 (d, J = 12 Hz, 1H); 4.86 (m, 1H); 6.91 (s, 1H); 6.94 (s, 1H); 7.2 (dd, J = 7.5, J = 8.4 Hz, 1H); 7.36 (bs, 1H). <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  24.6; 28.8; 50.7; 55.9; 61.3; 66.5; 74.8; 75.3; 111.7; 111.9; 119.1; 122.3; 126.3; 132.9; 152.7; 170.3 IR (Nujol): cm<sup>-1</sup> 3410, 2969, 1738, 1613, 1583, 1514, 1429, 1268, 1218, 1109, 1079, 1049, 809, 759. MS: m/e (relative intensity) 425 (M+, 10), 394 (20), 323 (30), 276 (35), 245 (100), 176 (100), 149 (45), 120 (40), 106 (20), 77 (30), 70 (100). HRMS Calculated for  $C_{16}H_{1}9C_{1}3N_{2}O_{5}$ : 424.0357. Found: 424.0359. [a]  $^{25}_{p}=-45.1^{\circ}$ 

 $(c = 0.63, CHCl_3).$ 

(11S,11aS)-11-hydroxy-9-methoxy-10-N-(2',2',2'-trichloroethoxycarbonyl)-1,2,-3,10,11,11a-hexahydro-5H-pyrrolo
[2,1-c][1,4] benzodiazepin-5-one (100)

A solution of DMSO (0.46 ml, 6.63 mmol) in of dry dichloromethane 5 (10 mL) was added drop wise over 30 minutes to a solution oxalyl chloride (3.30 mmol,) in dry dichloromethane (11.65 mL) at -40°C. The mixture was allowed to stir for a further 30 minutes, a solution of 99 (1 g, 2.37 mmol) in dichloromethane (15 mL) was then added drop wise over 1hour. Following the end of addition 10 the mixture was allowed to stir at -45°C for 60 minutes, then a solution of TEA (1.31 mL) in dichloromethane (6 mL) was added drop wise and the mixture was allowed to warm to room temperature. The reaction mixture was washed with water (50 mL), 1N HCl (2 x 25 mL), satd. aqueous NaHCO3 (2 x 25 mL), and brine 15 (50 mL). The organic solution was dried over  $MgSO_4$  and evaporated. The crude product was purified by flash chromatography (silica gel EtOAc/petroleum ether 1/1) to give a colourless oil (0.64 g, 63%):  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  2.01 -2.15 (m, 4H); 3.43 - 3.58 (m, 2H); 3.73 (m, 2H); 3.83 (s, 3H); 20 4.35 (d, J = 12, 1H); 4.98 (d, J = 12, 1H); 5.66 (dd, J = 3.8, J =9.6 Hz, 1H); 7.02 (dd, J = 2.2, J = 7.5 Hz, 1H); 7.35 (m, 2H).  $^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  23.0; 28.6; 46.2; 56.1; 59.9; 75.3; 86.2; 94.8; 113.4; 120.2; 123.1; 129.4; 134.9; 154.7; 155.4; 166.7. IR (Nujol): cm<sup>-1</sup> 3291, 2924, 1724, 1616, 1580, 1463, 25 1318, 1278, 1075, 945, 812, 739. MS: m/e (relative intensity)

422 (M-1, 40), 387 (3), 275 (10), 245 (15), 217 (10), 176 (100),

150 (8), 120 (6), 70 (95). HRMS Calculated for  $C_{16}H_17C_13N_2O_5$ : 422.0202. Found: 422.0203. [a]  $^{25}_{p}$  + 136.5° (c = 0.19, CHCl3).

(11aS)-9-methoxy-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1c][1,4]benzodiazepin-5-one (101)

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Finely ground Cd/Pb couple (1.02 g). was added in small portions 5 to a stirred solution of 100 (0.64 g, 1.51 mmol) in THF (10 mL) and 1M NHAOAc (10 mL). The reaction was followed by TLC (EtOAc), when no more starting material was observed, the mixture was poured into ethyl acetate (200 mL). The organic phase was dried over  $MgSO_4$  and evaporated to yield the product as a pale yellow oil (0. 28 g, 80%):  $^{1}$ H NMR (270MHz, CDCl<sub>3</sub>)  $\delta$  2.15 (m, 4H); 3.52 (m, 2H); 3.87 (s, 3H); 5.15 (m, 1H); 6.8 - 7.2 (m, 3H); 7.8 (d, J)= 4.7 Hz, 1H, imine H11). IR (Nujol): cm<sup>-1</sup> 3373, 2975, 1621, 1576, 1440, 1419, 1250, 1075, 750. MS: m/e (relative intensity) 230  $(M^+\cdot, 100)$ , 215 (45), 201 (20), 187 (5), 160 (5), 146 (4), 133 (20), 105 (10), 76 (25), 70 (45), 63 (3), 51 (3). HRMS Calculated for  $C_{13}H_14N_2O_2$ : 230.1055. Found: 230.1055.  $455.3^{\circ}$  (c = 0.6, CHCl<sub>3</sub>).

# Example 3(d): Synthesis of the 7.8-Dimethoxy PBD (106, AG/105) (see Figure 14)

4,5-dimethoxy-2-(2',2',2'-trichloroethoxycarbonylamino)benzoic acid (103)

A solution of Troc-Cl (0.76 ml, 5.56 mmol) in dry dichloromethane (10 mL) was added dropwise to 2-amino-4,5-dimethoxybenzoic acid 102 (1 g, 5.1mmol) and pyridine (0.82 ml, 10.1 mmol) in dry dichloromethane (20 ml) at 0°C. The reaction mixture was allowed to stir overnight at room temperature and then washed with dilute HCl (1N, 2 x 2 5 ml), water (2 x 25 ml) and brine (20 ml). The organic phase was dried over MgSO<sub>4</sub> and evaporated to yield of crude product (1.6 g), which was used in the next step without further purification.

N-(4,5-dimethoxy-2'-(2",2",2"-

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trichloroethoxycarbonylamino)benzoyl)-pyrrolidine-2-methanol (104)

Oxalyl chloride (0.38 mL, 4.33 mmol) was added to the crude Trocprotected anthranilic acid, prepared in the previous reaction,
together with 2 drops of dry DMF in dry dichloromethane (30 mL).

After initial strong effervescence, the mixture was allowed to
stir at room temperature overnight. The resulting acid chloride
was added dropwise, over 30 minutes, to a solution of 2S-(+)pyrrolidinemethanol (0.44 g, 4.33 mmol) and TEA (1.37 ml, 9.85

mmol) of dry dichloromethane (15 mL) at -16°C. The reaction mixture was diluted with ethyl acetate (20 mL), and washed with dilute HCl (1N, 2 x 30 mL), satd. aqueous NaHCO3 (2 x 30 mL), water (30 mL) and brine (30 mL). The organic layer was then dried over MgSO<sub>4</sub> and evaporated to give a yellow oil. The crude product was purified by flash chromatography (petroleum ether/ethyl acetate = 50/50) to yield the product (1.2 g, 70%) as a pale yellow oil:  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  1.75 (m, 2H); 1.92 (m, 1H); 2.17 (m, 1H); 3.53 (m, 2H); 3.72 (m, 1H); 3.86 (s, 3H); 3.93 (s, 3H); 4.19 (m, 1H); 4.43 (m, 1H); 4.77 (d, J = 12Hz, 1H); 4.85 (d, J = 12 Hz, 1H); 6.85 (s, 1H); 7.69 (s, 1H); 9.08 (bs, 1H).  $^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  25.1; 28.2; 51.4; 56.0; 56.4; 60.8; 65.9; 74.4; 95.3; 104.7; 110.7; 116.3; 130.8; 144.4; 151.0; 152.1; 170.4. MS: m/e (relative intensity) 454 (M-1, 5), 356 (3), 306 (10), 275 (5), 206 (100), 179 (15), 150 (10), 136 (3), 70 (45). HRMS Calculated for  $C_{17}H_21C_13N_2O_6$ : 454.0465. Found: 454.0464. [a]  $^{25}_{p} = -72.2$ ° (c = 0.18, CHCl3).

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(11S,11aS)-7,8-dimethoxy-11-hydroxy-10-N-(2',2',2'-trichloroethoxycarbonyl)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo
[2,1-c][1,4] benzodiazepin-5-one (105)

A solution of DMSO (0.9 ml, 12.9 mmol) in dry dichloromethane (15 mL) was added dropwise over 30 minutes to a solution of oxalyl chloride (6.4 mmol) of dry dichloromethane (15 mL) keeping the temperature below -40°C. The reaction mixture was allowed to stir for further a 30 minutes at which point a solution of 104 (2.1 g, 4.61 mmol) in dichloromethane (35 mL) was added drop wise over 1 hour. After addition of the substrate the reaction

mixture was allowed to stir at -45°C for 60 minutes, and then treated with a solution of TEA (2.56 mL) in of dichloromethane (10 mL) were added drop wise and the mixture was allowed to warm to room temperature. The reaction mixture was washed with water (75 mL), dilute HCl (1N, 75 mL), water (75 mL), brine (75 mL) 5 dried over MgSO<sub>4</sub> and evaporated. The crude product was purified by flash chromatography (EtOAc/petroleum ether 40/60) to give a colourless oil (1.19 g, 57%):  ${}^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  2.04 (m, 2H); 2.11 (m, 2H); 3.47 - 3.59 (m, 2H); 3.68 - 3.75 (m, 1H); 3.91 (s, 3H); 3.94 (s, 3H); 4.21 (d, J = 12.1 Hz, 1H); 4.43 (d, J = 12.1 Hz, 1H);10 4.76 Hz, 1H); 5.27 (d, J = 12.1 Hz, 1H); 5.65 - 5.7 (dd, J =4.58, J = 9.71 Hz, 1H); 6.82 (s, 1H); 7.26 (s, 1H). <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  23.1; 28.6; 46.4; 56.0; 56.1; 60.0; 74.9; 86.4; 95.1; 110.3; 112.7; 125.6; 148.6; 150.8; 154.5; 167.0. MS: m/e (relative intensity) 452 (M-1, 30), 424 (7), 354 (10), 276 15 (25), 206 (100), 180 (10), 150 (10), 70 (100). HRMS Calculated for  $C_{17}H_{1}9Cl_{3}N_{2}O_{6}$ : 452.0308. Found: 452.0309. [a]<sup>25</sup> = + 104.7°  $(c = 0.27, CHCl_3).$ 

(11aS)-7,8-dimethoxy-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (106, AG/105)

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Finely ground Cd/Pb couple (3.12 g) was added portion wise to a solution of 105 (1 g, 2.2 mmol) THF (10 mL) and NH<sub>4</sub>OAc (1M, 10 mL). The reaction was followed by TLC (EtOAc), when no starting material was present, the mixture was poured into ethyl acetate (400 mL). The organic phase was dried over MgSO<sub>4</sub> and evaporated to yield the crude product, which was purified by flash chromatography (EtOAc) to give of the pure compound as a pale

yellow oil (0.45 g, 78%): <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  2.08 (m, 2H); 2.29 (m, 2H); 3.53 - 3.63 (m, 1H); 3.72 (m, 1H); 3.79 - 3.85 (m, 1H); 3.93 (s, 3H); 3.96 (s, 3H); 6.82 (s, 1H); 7.52 (s, 1H); 7.68 (d, J = 4.4, 1H). <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  24.2; 29.6; 46.7; 53.7; 56.0; 56.1; 109.4; 111.2; 140.7; 147.5; 151.3; 162.5; 164.6. IR (Nujol): cm<sup>-1</sup> 3000-2800, 1601, 1450, 1434, 1500, 1453, 1263, 1217, 1010, 908, 735. MS: m/e (relative intensity) 260 (M+, 100), 245 (50), 231 (25), 217 (10), 191 (20), 164 (25), 136 (20), 121 (5), 93 (8), 70 (10). HRMS Calculated for C<sub>14</sub>H<sub>1</sub>6N<sub>2</sub>O<sub>3</sub>: 260.1160. Found: 260.1161. [a] <sup>25</sup><sub>D</sub> = + 1004.7° (c = 0.17, CHCl<sub>3</sub>).

# Example 3(e): Synthesis of the 6,7,8-Trimethoxy PBD (113, DRH-NA7) (see Figure 15)

## 2,3,4-Trimethoxy-6-nitrobenzoic acid (108)

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2,3,4-trimethoxybenzoic acid 107 (25 g, 117.8 mmol) was added portionwise to a stirred solution of 70% nitric acid at 0°C for 30 minutes. The reaction mixture was poured into cold water (1250 mL) and stirring was continued for 30 minutes. The reaction mixture was extracted with EtOAc (2 x 200 mL) and the combined organic layers were washed with brine (2 x 200 mL) and dried over anhydrous MgSO4. Evaporation of excess solvent in vacuo afforded the product as a pure white crystalline solid (18.67 g, 60%):  $R_f = 0.5$  (silica, EtOAc); IR (nujol) 2922, 1713, 1618, 1570, 1504, 1464, 1401, 1308, 1246, 1168, 1111, 1028, 920,

852, 789, 773, 728, 689 cm<sup>-1</sup>; <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.76 (1H, s), 4.0 (3H, s), 3.95 (3H, s), 3.90 (3H, s); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  166.0, 153.2, 150.1, 147.79, 139.6, 120.8, 103.6, 62.2, 61.1, 56.5; MS (EI) m/z 258 (M+1), 240, 214.

5 N-(2-Nitro-4,5,6-trimethoxybenzoyl)pyrrolidine-2-methanol (109) A catalytic quantity of DMF (2 drops) was added to a stirred solution of 108 (10 g, 38.9 mmol) and oxalyl chloride (5.87 g, 46.2 mmol) in dry CHCl<sub>2</sub> (100 mL) under a nitrogen atmosphere. The reaction mixture was allowed to stir overnight, and the 10 product was used directly in the next stage of the reaction. newly formed acid chloride was added dropwise to a stirred solution of pyrrolidinemethanol (3.92 g, 38.8 mmol) and anhydrous triethylamine (12.4 mL, 9.8 g, 97.0 mmol) in anhydrous DCM (50 mL) at 0°C under nitrogen. Once the addition was complete, the reaction mixture was left to warm to room temperature and left to 15 stir overnight. The reaction mixture was washed with 1N HCl (100 mL), water (100 mL), and brine (2 x 100 mL). The combined organic layers were dried (MgSO<sub>4</sub>) and the solvent was removed in vacuo to afford 109 (12.1 g, 91%) as a pale yellow oil:  $R_f = 0.39$  (silica, EtOAc); [a]  $^{21.9}$  p +135° (c = 0.1, DCM); IR (neat) 3400, 3105, 20 2947, 2878, 1652, 1568, 1538, 1455, 1348, 1250, 1195, 1115, 975, 922, 849, 822, 792, 758, 733, 646 cm<sup>-1</sup>;  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.59 (1H, s), 4.46 (2H, d, J = 2.93 Hz), 4.07 (3H, s), 4.03 (3H, s), 4.01 (3H, s), 3.89 (3H, t), 3.45-3.29 (2H, m), 2.24-2.17 (2H, m), 2.00-1.84 (2H, m);  $^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>, rotamers)  $\delta$ 25 165.7, 165.1, 153.3, 149.2, 148.1, 138.8, 122.5, 104.1, 66.4,

65.5, 62.4, 62.3, 61.3, 56.6, 49.2, 49.0, 28.7 24.3; MS (EI) m/z

341 (M+1), 324, 309, 293, 277, 264, 254.

N-(2-Amino-4,5,6-trimethoxybenzoyl)pyrrolidine methanol (110) Hydrazine hydrate (5.67 g, 177.2 mmol) was added dropwise to a solution of 109 (12.1 g, 35.47 mmol) in gently refluxing methanol (142 mL) over Raney nickel (3.45 g, slurry). The resulting 5 vigorous evolution of hydrogen gas subsided after approximately 10 mins and the reaction was deemed to be complete by TLC after 3 h. The reaction mixture was filtered through celite and the solvent evaporated. Distilled water (200 mL) was added to the residue, and the aqueous mixture was extracted with DCM (2 x 100  $\,$ 10 mL) and the combined organic phase washed with  $H_2O$  (3 x 100 mL) and brine (3 x 100 mL) and dried over anhydrous  $MgSO_4$ . Evaporation of the solvent afforded 110 (11.24 g) as a yellow oil.  $R_f = 0.14$  (silica, EtOAc); [a]  $^{21.8}$  D = +100° (c = 0.1, DCM); IR (neat)  $cm^{-1}$  3355, 2940, 2879, 2843, 1614, 1498, 1463, 15 1428, 1410, 1365, 1339, 1240, 1199, 1123, 1078, 1039, 997, 915, 817, 731, 646;  $^{1}\text{H}$  NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  6.10 (1H, s), 4.37 (2H, d, J = 3.67 Hz), 3.93 (3H, s), 3.88 (3H, s), 3.86 (3H, s), 3.67 (2H, t), 2.17-2.02 (2H, m), 1.87-1.82 (2H, m)  $^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  168.8, 154.7, 150.9, 149.6, 140.6, 133.8, 95.8, 66.5, 20 61.8, 61.4, 61.3, 61.1, 49.2, 28.6, 24.4; MS (EI) m/z 310 (M+), 294, 279, 229, 210, 194, 180, 149, 124, 102, 83, 70, 57.

N-(2-[2',2',2'-Trichloroethoxycarbonylamino]-4,5,6trimethoxybenzoyl)pyrrolidine-2-methanol (111)

25 A stirred solution of 110 (11.24 g, 36.3 mmol) in DCM (150 mL)

and pyridine (5.86 mL, 5.73 g, 72.5mmol) was treated dropwise with 2,2,2-trichloroethyl chloroformate (5 mL, 7.61 g, 35.9 mmol) in DCM (50 mL) under a nitrogen atmosphere at 0°C. One hour after the addition of 2,2,2-trichloroethyl chloroformate, the reaction mixture was diluted with DCM (100 mL) and washed with 1N HCl (100 mL), water (2 x 150 mL), brine (2 x 100 mL) and dried (MgSO<sub>4</sub>). The solvent was removed in vacuo to afford 111 (15.44 g, 88%) as a clear brown oil:  $R_f = 0.44$  (silica, EtOAc); IR (neat) cm<sup>-1</sup> 3437, 2948, 1738, 1628, 1497, 1458, 1422, 1397, 1238, 1115, 1027, 1008, 823, 760, 624;  $^1$ H NMR (270 MHz, DMSO)  $\delta$  6.82 (1H, s), 5.06 (2H, s), 4.04 (2H, d, J = 6.83 Hz), 3.85 (3H, s), 3.84 (3H, s), 3.79 (3H, s), 3.67 (2H, t), 2.00-1.97 (2H, m), 1.96-1.88 (2H, m)  $^{13}$ C NMR (67.8 MHz, DMSO)  $\delta$  164.2, 153.5, 149.6, 139.6, 129.4, 121.3, 96.2, 73.9, 61.4, 60.9, 58.7, 56.2, 47.9, 27.5, 23.7; HRMS (FAB) calcd for  $C_{18}$ H<sub>2</sub>3N<sub>2</sub>O<sub>7</sub>Cl<sub>3</sub> (M<sup>+·)</sup> 484.0571, found 484.0944.

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6,7,8-Trimethoxy-10-(2',2',2'-trichloroethoxycarbonyl)1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5one (112)

A solution of oxalyl chloride in DCM (22.3 mL of a 2N solution,
44.7 mmol) diluted with anhydrous DCM (42 mL) at -45°C was
treated dropwise with a solution of anhydrous DMSO (6.39 mL, 90.2
mmol) in anhydrous DCM (16.24 mL) over a period of 15 minutes.
The reaction mixture was stirred at -45°C for 15 minutes and
treated with a solution of 111 (15.44 g, 31.7 mmol) in dry DCM

(34.3 mL) and stirred at -45°C for 45 minutes. Triethylamine
(17.7 mL, 127.1 mmol) was added dropwise to the reaction mixture
over 0.5 h, and then allowed to stir for a further 15 minutes.

The reaction mixture was allowed to warm to room temperature and diluted with water (100 mL). The organic layer was washed with 1N HCl (200 mL), water (200 mL), brine (200 mL) and dried (MgSO<sub>4</sub>). The reaction mixture was evaporated and purified by 5 flash column chromatography (EtOAc) to afford the product 112 (8.27 g, 54%) as a clear yellow glass:  $R_f = 0.48$  (silica, EtOAc); [a]  $^{22.2}$  D +190° (c 0.15, DCM); IR (neat) cm<sup>-</sup>1 3262, 2979, 2943, 2885, 1732, 1613, 1493, 1456, 1399, 1372, 1334, 1299, 1264, 1244, 1201, 1118, 1059, 1014, 969, 926, 888, 838, 784, 756, 720, 693, 624; <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  6.64 (1H, s), 5.58 (1H, s), 10 5.31 (1H, s), 4.34 (1H, d, J = 19.78 Hz), 4.15-4.00 (1H, m), 3.95 (3H, s), 3.91 (3H, s), 3.90 (3H, s), 3.77 (2H, t), 3.55 (1H, t), 2.17-2.14 (2H, m), 2.14-2.10 (2H, m). <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  163.49, 154.32, 152.30, 142.69, 129.51, 121.16, 109.35, 95.20, 85.63, 62.30, 61.36, 60.48, 56.09, 45.56, 28.44, 22.85; MS (EI) 15 m/z 485 (M+1), 467, 398, 384, 350, 291, 254, 236, 222, 194, 131, 102, 82, 70, 57.

6,7,8-Trimethoxy-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (113)

2939, 1614, 1455, 1428, 1392, 1359, 1275, 1245, 1203, 1113, 1052, 1035, 1000, 926, 804, 751, 665;  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  (1H, d, J = 4.39 Hz), 6.61 (1H, s), 6.14 (1H, d, J = 8.24 Hz), 4.36 (1H, d, J = 8.79 Hz), 4.01 (3H, s), 3.98 (3H, s), 3.84 (3H, s), 3.48-3.46 (2H, m) 2.26-2.23 (2H, m), 2.16-1.93 (2H, m); HRMS (FAB) calcd for  $C_{15}H_{1}8N_{2}O_{4}$  (M+1) 290.1266, found 290.1208.

# Example 3(f): Synthesis of the 7,8,9-Trimethoxy PBD (120, DRH-69) (see Figure 16)

## 3,4,5-Trimethoxy-2-nitrobenzoic acid (115)

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Methyl 3,4,5-trimethoxy-2-nitrobenzoic 114 (24.37 g, 89.9 mmol) was added to a 5% solution of KOH (18 g) in MeOH (357 mL). The mixture was heated at reflux for 50 minutes. Evaporation of the solvent afforded a grey residue, which was dissolved in H<sub>2</sub>O (200 mL) The resulting alkaline solution was acidified to pH1 with concentrated HCl, and extracted with CHCl<sub>3</sub> (3 x 100 mL). The organic layer was washed with H<sub>2</sub>O (3 x 100 mL), brine (3 x 100 mL) and dried over anhydrous MgSO<sub>4</sub>. Filtration and evaporation of the solvent afforded a pure white crystalline solid (20.67 g, 80.4 mmol): <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) δ 3.9 (s, 3H), 4.0 (s, 3H), 4.1 (s, 3H), 7.4 (s, 1H), 12.4 (br s, 1H).

N-(2-Nitro-3,4,5-trimethoxybenzoyl)pyrrolidine-2-methanol (116) A catalytic amount of DMF (2 drops) was added to a stirred solution of 115 (2.57 g, 10 mmol) and oxalyl chloride (1.40 g, 11 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (40 mL) under an inert atmosphere. 5 reaction mixture was allowed to stir overnight, the resulting solution of the acid chloride, (2.76 g, 10 mmol) in anhydrous CH2Cl2 (40 mL) was added dropwise over 1 h to a vigorously stirred solution of pyrrolidinemethanol (1.11 g, 11 mmol) and TEA (2.52 g, 25 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (40 mL) under a nitrogen atmosphere at 0°C and allowed to stir overnight at room 10 temperature. The reaction mixture was washed with 1N HCl (1 x 50 mL), 1N NaOH (1 x 50 mL),  $H_2O$  (3 x 50 mL) and brine (3 x 50 mL) and dried over anhydrous MgSO<sub>4</sub>. Filtration and evaporation of the solvent afforded a yellow oil (2.81 g, 8.3 mmol): Rf = 0.47(5% MeOH/CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  1.7-2.0 (m, 3H), 2.1-15 2.2 (m, 1H), 3.3-3.5 (m, 2H), 3.7-3.9 (m, 2H), 3.9-4.0 (2 x s, 6H), 4.0-4.1 (s, 3H), 4.2-4.3 (m, 1H), 6.7 (s, 1H); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  167.3, 156.5, 147.9, 143.5, 128.8, 104.8, 65.8, 62.6, 61.4, 61.2, 56.6, 50.2, 28.4, 28.1, 24.5, 14.2.

N-(2-Amino-3,4,5-trimethoxybenzoyl)pyrrolidine-2-methanol (117)

Hydrazine hydrate (1.33 mL, 41.5 mmol) was added dropwise to a solution of 116 (2.83 g, 8.3 mmol) in methanol (142 mL) gently refluxing over Raney nickel (500 mg, slurry). The resulting vigorous evolution of hydrogen gas subsided after approximately 10 minutes and the reaction was deemed to be complete by TLC after 2 h. The reaction mixture was filtered through celite and the solvent evaporated. Distilled water (100 mL) was added to

the residue, and the aqueous mixture was extracted with EtOAc (3 x 100 mL) and the combined organic phase washed with  $H_2O$  (3 x 100 mL) and brine (3 x 100 mL) and dried over anhydrous  $MgSO_4$ . Evaporation of the solvent afforded the product (2.18 g, 6.5 mmol) as a brown oil:  $^1H$  NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  1.6-2.0 (m, 3H), 2.1-2.2 (m, 1H), 3.4-3.7 (m, 4H), 3.8 (s, 3H), 3.8-3.9 (2 x s, 6H), 4.4 (br s, 1H), 4.7-4.3 (br s, 1H), 6.6 (s, 1H);  $^{13}C$  NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  144.7, 144.5, 141.6, 134.6, 107.1, 66.9, 61.0, 60.9, 60.5, 56.8, 50.9, 28.6, 24.9, 21.1, 14.2.

# N-2-(Trichloroethoxycarbonylamino)-3,4,5trimethoxybenzoyl)pyrrolidine-2-methanol (118)

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A solution of 2,2,2-trichloroethylchloroformate (1.37 g. 6.5 mmol) in distilled dichloromethane (40 mL) was added dropwise over 0.5 h to a solution of anhydrous pyridine (0.93 g, 11.8 15 mmol) and the substrate, 117 (1.82 g, 5.9 mmol) in distilled dichloromethane (60 mL) at 0°C. After 1.5 h. the reaction mixture was diluted with anhydrous DCM (100 mL) and washed with 1N HCl (2 x 100 mL),  $H_2O$  (100 mL), brine (100 mL) and dried over anhydrous MgSO4. Evaporation of the solvent yielded a brown oil which was purified by flash column chromatography eluting with 1% 20 MeOH/ 99% CHCl3 to afford the product as a yellow oil (1.83 g, 3.8 mmol):  ${}^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  1.6-1.9 (m, 3H), 2.1-2.2 (m, 1H), 3.3-3.6 (m, 2H), 3.6-3.85 (m, 2H), 3.8-3.9 (m, 9H), 4.2-4.3 (m, 1H), 4.7-4.8 (br s, 1H), 4.8 (s, 2H), 6.6 (s, 1H);  $^{13}$ C NMR  $(67.8 \text{ MHz}, \text{CDCl}_3)$   $\delta$  169.9, 153.2, 151.9, 143.1, 128.5, 120.1, 25 105.2, 95.3, 74.6, 66.3, 61.2, 61.2, 61.0, 56.3, 50.6, 28.7, 24.6.

(11S,11aS)7,8,9-trimethoxy-11-hydroxy-10-N-(2',2',2'-trichloroethoxycarbonyl)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4] benzodiazepin-5-one (119)

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Anhydrous DMSO (3.15 mL, 44.3 mmol) in dry DCM (8.2 mL) was added dropwise over 20 mins to a stirred solution of oxalyl chloride (2.79 q, 11.0 mL of a 2N solution in DCM; 22.0 mmol) in dry DCM (20.6 mL) under an inert atmosphere at -45° C (varied between -38° and -48°C). After stirring for 15 mins, the substrate (7.59g; 15.6 mmol) in dry DCM (17 mL) was added dropwise over 45 mins to the reaction mixture, which was then stirred for a further 45 mins at -45°C after the final addition of the substrate. Dry TEA (4.84 g, 48.0 mmol, 4 eq) was added dropwise to the mixture over 0.5 h and stirred for a further 15 mins. The reaction mixture was allowed to warm to room temperature and the reaction mixture diluted with  ${\rm H}_2{\rm O}$  (80 mL). The organic phase was separated, washed with brine (2 x 100 mL) and dried over anhydrous MgSO<sub>4</sub>. The solvent was evaporated to afford the product as an off-white solid (4.39 g, 9.1 mmol):  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  1.95-2.2 (m, 4H), 3.4-3.8 (m, 2H), 3.8-3.9 (m, 9H), 4.05 (d, 1H), 4.5-4.8 (dd, 2H), 5.6-5.7 (q, 1H), 7.1 (s, 1H);  $^{13}$ C NMR (CDCl<sub>3</sub>) rotamers  $\delta$ 166.7, 166.5, 155.2, 153.5, 153.3, 150.0, 144.5, 129.5, 129.0, 121.7, 106.4, 106.2, 94.6, 86.1, 85.9, 75.7 75.2, 61.5, 61.3, 60.9, 60.1 59.8, 56.2, 56.1, 46.5, 46.3, 28.7, 28.6, 23.0.

7,8,9-Trimethoxy-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (120, DRH-69)

10% Cd/Pb couple (1.25 g, 10 mmol Cd) was added to a rapidly stirring solution of the Troc-carbamate, 119 (1.00 g, 2.1 mmol)

in a mixture of THF (13 mL) and 1N NH<sub>4</sub>OAc (8 mL). Upon addition, the reaction mixture went cloudy. After 40 minutes, TLC showed the reaction to be complete and the reaction mixture was diluted with EtOAc (200 mL). The solution was dried over anhydrous MgSO<sub>4</sub> and the solids were filtered and rinsed with EtOAc (50 mL). Evaporation of the solvent yielded the product as a yellow glass (0.581 g, 2.0 mmol).  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.73 (d, 1H, J = 4.57 Hz), 7.08 (s, 1H), 4.0-3.4 (m, 12H), 2.4-1.8 (m, 4H)

# Example 3(g): 8-Hydroxy-7,9-dimethoxy-1,2,3,11a tetrahydropyrrolo[2,1-c][1,4]benzodiazepin-5-one (130, DRH 168) (see Figure 17)

## Methyl 4-hydroxy-3,5-dimethoxybenzoate (121)

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Concentrated sulphuric acid (3 mL), was added dropwise to a solution of 81 (20.24 g, 102.1 mmol) in refluxing methanol (70 mL). The reaction mixture was heated at reflux for a further 5 h and then cooled to room temperature and concentrated to a third of its original volume. The concentrate was poured onto crushed ice (c. 150 mL) and alowed to stand for 30 minutes. The aqueous mixture was extracted with ethyl acetate (3 x 100 mL) and the combined organic phase washed with distilled water (3 x 100 mL), brine (3 x 100 mL) and dried over anhydrous MgSO<sub>4</sub>. Removal of ecxess solvent under reduced pressure afforded the product as a yellow solid, 121 (18.39 g, 86.7 mmol;  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $^{5}$ 

3.9 (s, 3H), 3.95 (s, 3H), 3.975 (s, 3H), 6.1 (s, 1H), 7.3 (s, 2H);  $^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  166.9, 146.6, 139.2, 121.0, 106.6, 56.4, 52.1.

## Methyl 4-Benzyloxy-3,5-dimethoxybenzoate (122)

- Benzyl chloride (11.04 g, 86.9 mmol) was added to a stirred solution of 121 (19.22 g, 90.8 mmol) over  $K_2CO_3$  (6.59 g, 47.7 mmol) in anhydrous MeOH (175 mL) and the mixture was heated at reflux for 12 h. Excess solvent was removed under reduced pressure and the residue was extracted with benzene (3 x 100 mL).
- The organic layer was washed with  $\rm H_{2}O$  (3 x 100 mL), brine (3 x 100 mL) and dried over anhydrous MgSO<sub>4</sub>. Evaporation of the solvent afforded an orange oil which crystallised on standing. The solid was redissolved in EtOAc, and briefly washed with 1N NaOH (100 mL),  $\rm H_{2}O$  (100 mL), brine (100 mL) and dried over MgSO<sub>4</sub>.
- Evaporation of excess solvent yielded the product as a yellow solid 122 (19.20 g, 63.6 mmol); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) δ 3.8 (s, 3H), 3.85 (s, 3H), 3.9 (s, 3H), 5.1 (s, 2H), 7.3-7.5 (m, 7H); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>) δ 166.7, 153.2, 140.8, 137.3, 128.7, 128.6, 128.4, 128.4, 128.2, 128.0, 127.7, 125.3, 106.7, 74.9, 56.1,

# Methyl 2-nitro-4-benzyloxy-3,5-dimethoxybenzoate (123)

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Finely ground copper nitrate  $(Cu(NO_3)_2, 14.79 \text{ g}, 78.7 \text{ mmol})$  was added portionwise to a vigorously stirred solution of the substrate (19.00 g, 62.9 mmol) in acetic anhydride (120 mL) whilst keeping the reaction temperature below 40° C. The

reaction mixture was stirred for 1 h and then poured over ice (800 mL). The aqueous mixture was left to stir for 1 h and the product collected by filtration to afford a yellow solid (18.7 g); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) δ 3.85 (s, 3H), 3.95 (s, 3H), 3.96 (s, 3H), 5.19 (s, 2H), 7.3-7.5 (m, 6H); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>) δ 163.2, 154.3, 146.0, 145.2, 136.2, 128.7, 128.5, 128.4, 128.3, 117.8, 108.52, 75.5, 62.7, 56.5, 53.0.

## 2-Nitro-4-benzyloxy-3,5-dimethoxybenzoic acid (124)

Potassium hydroxide (10.84 g, 193.6 mmol) was added to a stirred solution of the substrate (18.7 g, 53.9 mmol) in anhydrous methanol (220 mL) and the reaction mixture heated at reflux for 2 h. The reaction mixture was allowed to cool and acidified to pH2 with 1N HCl and extracted with chloroform (3 x 100 mL). The combined organic layers were washed with water (3 x 200 mL), brine (3 x 200 mL) and dried over MgSO<sub>4</sub>. Evaporation of excess solvent by rotary evaporation under reduced pressure afforded the product as a yellow solid (17.01 g, 51.1 mmol, 95%); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) δ 3.9 (br s, 3H), 3.9 (br s, 3H), 5.1 (br s, 2H), 7.2-7.5 (m, 6H).

# 20 N-(4-Benzyloxy-3,5-dimethoxy-2-nitrobenzoyl)pyrrolidine-2-methanol (125)

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A catalytic amount of DMF (5 drops) was added to a stirred solution of 124 (10g, 30.0 mmol) and oxalyl chloride (4.65 g, 36.0 mmol) in dry CH<sub>3</sub>CN (115 mL) under a nitrogen atmosphere. The reaction mixture was allowed to stir overnight and the

resulting acid chloride used directly in the next part of the procedure. 4-benzyloxy-3,5-dimethoxy-2-nitro-benzoyl chloride in anhydrous CH3CN (115 mL) was added dropwise over 0.5 h to a stirring solution of pyrrolidine methanol (3.34 g, 33.03 mmol, 1.1 eq) and TEA (7.58 g, 75.1 mmol, 2.5 eq) in anhydrous DCM (100 mL) at 0° C under a nitrogen atmosphere and the reaction mixture was allowed to stir overnight at room temperature. The reaction mixture was washed with 1N HCl (2 x 100 mL), and the organic layer was washed with distilled H2O (2 x 100 mL), brine (2 x 100 mL) and dried over anhydrous MgSO<sub>4</sub>. Evaporation of the solvent yielded a brown glass  $(8.71 \text{ g}, 20.9 \text{ mmol}, 70\%); ^1\text{H} NMR (270 MHz,$ CDCl<sub>3</sub>)  $\delta$  1.7-2.2 (m, 4H), 3.3-3.5 (m, 2H), 3.7-3.9 (m, 2H), 3.9 (s, 3H), 4.0 (s, 3H), 4.2-4.3 (m, 1H), 5.1 (s, 2H), 6.85 (s, 1H), 7.3-7.5 (m, 5H);  $^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  167.3, 156.8, 148.2, 142.3, 136.4, 136.0, 129.0, 128.5, 128.4, 104.8, 75.6, 65.7, 62.8, 61.4, 56.6, 50.2, 28.3, 24.5.

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# N-(2-Amino-4-Benzyloxy-3,5-dimethoxybenzoyl)pyrrolidine-2-methanol (126)

Hydrazine hydrate (2.31 g, 72.2 mmol) was added dropwise to a solution of 125 (6.01 g, 14.4 mmol) in methanol (60 mL) gently refluxing over Raney nickel (1.1g, slurry). The resulting vigorous evolution of hydrogen gas subsided after approximately 10 minutes and the reaction was deemed to be complete by TLC after 2 h. The reaction mixture was filtered through celite and the solvent evaporated. Distilled water (100 mL) was added to the residue, and the aqueous mixture was extracted with EtOAc (3 x 100 mL) and the combined organic phase washed with  $\rm H_{2}O$  (3 x 100

mL) and brine (3 x 100 mL) and dried over anhydrous MgSO<sub>4</sub>. Evaporation of the solvent afforded the produc as a brown oil (3.97 g, 10.3mmol, 73%):  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  1.6-2.2 (m, 4H), 3.5-3.8 (m, 4H), 3.8 (s, 3H), 3.9 (s, 3H), 4.4 (br s, 1H), 5.1 (s, 2H), 6.6 (s, 1H), 7.3-7.6 (m, 5H);  $^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  171.5, 144.9, 143.5, 141.9, 137.5, 134.6, 128.6, 128.5, 128.3, 128.2, 128.0, 115.1, 107.3, 75.1, 66.9, 61.0, 60.6, 60.4, 56.9, 50.9, 28.5, 24.9, 21.1, 14.2.

N-(4-Benzyloxy-3,5-dimethoxy-2-[(2'-

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trimethylsilylethoxy)carbonylamino[benzoyl)pyrrolidine-2-methanol
(127)

A solution of anhydrous pyridine (0.21 g, 2.6 mmol) in anhydrous DCM (10 mL) was added dropwise over 15 minutes to a stirred solution of 2-(trimethylsilyl)ethanol (0.92 g, 7.8 mmol) and triphosqene (0.77 g, 2.6 mmol) in anhydrous DCM (30 mL). 15 reaction mixture was allowed to stir overnight and the resulting solution of 2-(trimethylsilyl)ethyl chloroformate added dropwise over 0.5 h to the amine 126 (1.98 g, 5.1 mmol) and anhydrous pyridine (1.22 q, 15.4 mmol) in distilled dichloromethane (70 mL) at 0°C. The reaction mixture was allowed to stir overnight at 20 room temperature, diluted with anhydrous DCM (100 mL), washed with 1N HCl (3 x 100 mL),  $H_2O$  (3 x 100 mL), brine (3 x 100 mL) and dried over anhydrous MgSO4. Filtration and evaporation of the solvent yielded the product as a colourless glass (1.43 g, 25 2.7 mmol, 53%);  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  -0.05 (s, 9H), 0.94-0.99 (m, 2H), 1.66-2.12 (m, 4H), 3.32-3.54 (m, 2H), 3.74-3.88 (m, 8H), 4.05-4.22 (m, 3H), 4.69 (br s, 1H), 4.97 (s, 2H), 6.57 (s,

1H), 6.64 (br s, 1H), 7.23-7.43 (m, 5H); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>) δ 170.1, 155.1, 151.4, 148.1, 142.0, 137.1, 128.4, 128.3, 128.1, 121.2, 105.6, 75.3, 66.1, 64.0, 61.3, 61.0, 56.3, 50.6, 28.7, 24.7, 17.6, -1.5.

(11S, 11aS) -8-benzyloxy-7, 9-dimethoxy-11-hydroxy-10-N-(2'-5 trimethylsilylethoxycarbonyl)-1,2,3,10,11,11a-hexahydro-5Hpyrrolo[2,1-c][1,4] benzodiazepin-5-one. (128) Anhydrous DMSO (0.57 g, 7.2 mmol) in dry DCM (5 mL) was added dropwise over 30 minutes to a stirred solution of oxalyl chloride (0.46 g, 3.6 mmol) in dry DCM (5 mL) under a nitrogen atmosphere 10 at -45°C. After stirring for 15 minutes, the substrate (1.35 g, 2.6 mmol) in dry DCM (15 mL) was added dropwise over 45 minutes to the reaction mixture, which was then stirred for a further 45 minutes at -45°C. TEA (1.0 g, 10.2 mmol) was added dropwise to the mixture over 0.5 h and stirred for a further 15 mins. 15 reaction mixture was left to warm to room temperature and diluted with  ${\rm H}_2{\rm O}$  (100 mL) and the phases separated. The organic phase was washed with 1N HCl (3  $\times$  50 mL), water (3  $\times$  50 mL), brine (3  $\times$ 50 mL) and dried over MgSO<sub>4</sub>. Filtration and evaporation of excess solvent afforded the product as an off-white glass (1.24 20 g, 2.3 mmol, 92%);  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  -0.05 (s, 9H), 0.88-0.95 (m, 2H), 2.06-2.23 (m, 4H), 3.46-3.64 (m, 2H), 3.75-4.02 (m, 7H), 4.11-4.27 (m, 2H), 5.13 (s, 2H), 5.65 (d, 1H, J =9.71 Hz), 7.11 (s, 1H), 7.34-7.54 (m, 5H);  $^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  166.8, 157.2, 153.1, 150.5, 143.4, 137.1, 129.2, 128.4, 25 128.3, 128.3, 128.1, 123.0, 106.2, 85.7, 75.0, 64.7, 61.7, 59.8, 56.1, 46.4, 28.6, 23.0, 17.5, -1.5, -1.6.

(11S,11aS)-8,11-dihydroxy-7,9-dimethoxy-10-N-(2'-trimethylsilylethoxycarbonyl)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4] benzodiazepin-5-one(129)

10% Pd/C catalyst (0.22 g) was added to a solution of the

substrate 128 (0.95g, 2.1 mmol) in absolute EtOH (200 mL). The
reaction mixture was hydrogenated under pressure using a Parr
hydrogenator at 55 psi H<sub>2</sub> for 18 h. The reaction mixture was
filtered through celite, and the celite washed with hot EtOH,
taking care not to allow the filtration pad to dry out. Removal

of excess solvent afforded the product as a colourless glass
(0.84 g, 1.9 mmol, 92%); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) δ 0.07 (s, 9H),
0.91-0.97 (m, 2H), 2.07-2.20 (m, 4H), 3.52-3.75 (m, 2H), 3.984.26 (m, 9H), 5.65 (d, 1H, J = 9.71 Hz), 6.26 (br s, 1H), 7.14
(s, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 167.0, 157.3, 146.8, 143.4, 141.3,

15 124.9, 123.5, 105.5, 105.2, 85.8, 64.8, 64.6, 64.5, 61.2, 60.0,
56.4, 46.4, 28.9, 28.7, 23.1, 23.0, 17.3, -1.3, -1.5, -1.7.

# 7,9-dimethoxy-8-Hydroxy-1,2,3,11a-tetrahydropyrrolo[2,1-c][1,4]benzodiazepin-2-one (130)

A solution of TBAF in THF (4.3 mL of a 1N solution, 4.3 mmol) was added to a rapidly stirred solution of 129 (0.37 g, 0.9 mmol) in THF (10 mL) and the reaction mixture heated to 35° C for 2 h. The reaction mixture was diluted with EtOAc (50 mL), dried over anhydrous MgSO<sub>4</sub>, filtered and removal of excess solvent by rotary evaporation under reduced pressure afforded the product as a brown oil (0.18 g, 0.7 mmol, 78%). H<sup>1</sup> NMR (CDCl<sub>3</sub>) mixture of C11/C11'R/S carbinolamine methyl ethers δ 7.08 (s, 1H), 4.43 (d, 1H, J = 8.79 Hz), 4.05-3.23 (m, 12H), 2.3-1.48 (m, 4H).

## Examples 3(h) to (j): Synthesis of 7-Phenyl PBDs (See Figure 18)

Synthesis of the 7-Iodo-N10-Troc-PBD Intermediate (134, AG/91)
5-Iodo-2-(2',2',2'-trichloroethoxycarbonylamino)benzoic acid
(132)

- 5 A solution of Troc-Cl (2.88 mL, 20.9 mmol) in dry dichloromethane (20 mL) was added drop wise to a solution of 5-iodoanthranilic acid 131 (5 g, 19 mmol) and pyridine (3.1 mL, 38 mmol) in dry dichloromethane (30 mL) at 0°C. The solution was stirred for 5 hours at room temperature and then washed with 1N HCl (2  $\times$  25 10 mL), water (1 x 25 mL) and brine (1 x 25 mL). The organic phase was dried over MgSO4 and evaporated, residue was recrystallized from ethyl acetate to afford the title compound as a yellow solid (6.2 g, 75%): m.p. 248 C (ethyl acetate). <sup>1</sup>H NMR (CDCl<sub>3</sub>, DMSO $d_6$ )  $\delta$  4.83 (s, 2H); 7.78-7.82 (dd, J = 9.2, J = 2.2 Hz, 1H); 8.18 (d, J = 9 Hz, 1H); 8.38 (d, J = 2.2 Hz, 1H); 9.0-10.5 (bs, 1H);15 11.04 (s, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, DMSO- $d_6$ )  $\delta$  74.4, 84.6, 95.2, 117.7, 120.7, 140, 140.8, 142.8, 151.5, 169. MS: m/e (relative intensity) 437 (M-1, 60), 289 (55), 272 (37), 245 (100), 218 (27). HRMS Calculated for  $C_{1.0}H_7Cl_3INO_4$ : 436.8485. Found: 20 436.8485.
  - N-(5-Iodo-(2',2',2'-trichloroethoxycarbonylamino)benzoyl)
    pyrrolidine-2-methanol (133)

Oxalyl chloride (0.88 mL, 10 mmol) was added to a suspension of 132 (4 g, 9.1 mmol) in dry dichloromethane (50 mL), followed by 3-4 drops of DMF as catalyst. The solution was stirred at room

temperature for 12 hours, and then used directly in the next step. The newly formed acid chloride was added drop wise, over 1 hour, to a solution of 2S-(+)-pyrrolidinemethanol (1.01 q, 10 mmol) and triethylamine (3.16 mL, 22.7 mmol) in dry 5 dichloromethane (50 mL) at -20°C. The reaction mixture was allowed to stir for a further hour at -20°C and was then washed with dilute HCl (1N, 2 x 50 mL), water (50 mL) and brine (50 mL), dried over MgSO4 and evaporated. The crude product was subjected to flash column chromatography to afford the title compound as a 10 pale yellow oil (3.8 g, 81%):  ${}^{1}$ H NMR (CDCl<sub>3</sub>, DMSO- $d_6$ )  $\delta$  1.77-2.28 (m, 4H); 3.48 (bs, 2H); 3.7 (dd, J = 11.4, J = 6.2, 1H); 3.94 (d, J)J = 11.4 Hz, 1H; 4.40 (bs. 1H); 4.75 (d. J = 12 Hz., 1H); 4.84(d, J = 12 Hz, 1H); 7.66 - 7.72 (m, 2H); 7.85 (d, J = 8.6 Hz,1H); 8.91 (bs, 1H).  $^{13}$ C NMR (CDCl<sub>3</sub>, DMSO- $d_6$ )  $\delta$  25.0, 28.1, 51.2, 60.7, 65.3, 74.5, 86.1, 95.1, 123.0, 128.0, 135.6, 136.1, 139.8, 15 151.8, 168.4. IR (Nujol): cm<sup>-1</sup> 3415, 3215, 1745, 1605, 1527, 1445, 1377, 1221, 1101, 1056, 822, 733. MS: m/e (relative intensity) 522  $(M^{+}, 3)$ , 521  $(M^{+}, 1)$ , 520  $(M^{+}, 3)$ , 491 (3), 490 (1), 489 (3), 372 (7), 341 (28), 272 (80), 245 (14), 216 (14), 83 20 (15), 70 (100). HRMS Calculated for C<sub>15</sub>H<sub>1</sub>6Cl<sub>3</sub>IN<sub>2</sub>O<sub>4</sub>: 521.9193. Found: 521.9125. [a]  $^{25}_{D} = +123.4^{\circ}$  (c = 2.8, CHCl3).

7-Iodo-10-N-(2',2',2'-trichloroethoxycarbonyl)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzo- diazepin-5-one (134)

A solution of DMSO (1.79 mL, 25.67 mmol) in dry dichloromethane

(35 mL) was slowly added (30 minutes) to a solution of oxalyl chloride (12.8 mmol) in dry dichloromethane (41.4 mL) at - 45° C.

The mixture was allowed to stir for 25 minutes and then treated

with a solution of 133 (4.78 g, 9.2 mmol), in dry dichloromethane (80 mL), keeping temperature below -40° C. After further 60 minutes at -45° C, a solution of triethylamine (5.1 mL) in of dichloromethane (25 mL) was added, and the reaction mixture allowed to warm to room temperature. The organic phase was washed with water (180 mL), dilute HCl (1N,  $2 \times 100$  mL) and brine (200 mL). Removal of excess solvent afforded the crude product which was purified by flash chromatography (ethyl acetate/petroleum ether 70/30) to give of a pale yellow oil (3.6 g, 76%):  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  2.02-2.15 (m, 4H); 3.37-3.60 (m, 2H); 3.70-3.77 (m, 1H); 4.19 (bs, 1H); 4.28 (d, J = 12 Hz)1H); 5.17 (d, J = 12 Hz, 1H); 5.66 (d, J = 9.7 Hz, 1H); 7.10 (d, J = 8.3 Hz, 1H); 7.79 (dd, J = 8.3, J = 2.2 Hz, 1H); 8.10 (d, J =2.2 Hz, 1H).  $^{13}$ C NMR (CDCl<sub>3</sub>)  $\delta$  23.0, 28.8, 46.5, 59.6, 75.1, 86.0, 93.2, 94.8, 132.0, 133.6, 135.0, 137.9, 140.1, 154.1, 165.2. IR (Nujol): cm<sup>-1</sup> 3500-3000, 1716, 1619, 1458, 1376, 1312, 1075, 720. MS: m/e (relative intensity) 520 (M+, 62), 519 (22), 518 (62), 491 (15), 371 (19), 342 (39), 272 (84), 216 (31), 119 (27), 70 (100). HRMS Calculated for  $C_{15}H_14Cl_3IN_2O_4$ : 519.9036.

20 Found: 519.9037.  $[a]_{D}^{25} = +137.4^{\circ} (c = 1.15, CHCl3)$ .

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### Example 3(h): Synthesis of the 7-Phenyl-PBD (136, AG/129)

7-Phenyl-10-N-(2',2',2'-trichloroethoxycarbonyl)-1,2,3,10,11,11ahexahydro-5-H-pyrrolo[2,1-c][1,4] benzodiazepin-5-one (135) A suspension of 134 (0.5 g, 1.0 mmol), benzeneboronic acid (0.15 g, 1.22 mmol),  $Pd(PPh_3)_4$  and anhydrous  $Na_2CO_3$  (0.16 g, 1.48 mmol) 5 in distilled benzene (20 mL), water (2 ml) and ethanol (2 mL) was heated at reflux overnight. The reaction mixture was diluted with ethyl acetate (20 mL) and washed with water (2 x 20 mL). The organic phase was dried over MgSO<sub>4</sub> and evaporated to yield a 10 crude yellow oil. Purification by flash chromatography (ethyl acetate/petroleum ether 30/70 to 70/30) furnished the title compound (0.43 g, 95%):  ${}^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  1.98-2.09 (m, 2H); 2.12-2.15 (m, 2H); 3.51-3.62 (m, 2H); 3.7-3.79 (m, 1H); 4.28 (d, J = 12.1 Hz, 1H); 4.73 (d, J = 4.4 Hz, 1H); 5.18 (d, J = 12.1)Hz, 1H); 5.66-5.73 (dd, J = 4.8, J = 9.8 Hz, 1H); 7.33-7.48 (m, 15 4H); 7.61-7.70 (m, 3H); 8.02 (d, J = 2.2 Hz, 1H). <sup>13</sup>C NMR  $(CDCl_3)$   $\delta$  22.9, 28.7, 46.4; 59.8; 75.0; 77.3; 86.0; 94.9; 127.0; 127.3; 128.0; 128.9; 129.6; 130.8; 132.9; 133.5; 139.2; 141.1; 154.4; 166.9. MS: m/e (relative intensity) 468 (M<sup>+</sup>, 10), 292 (25), 222 (100), 195 (10), 166 (35), 140 (10), 70 (70). HRMS 20 Calculated for  $C_{21}H_19C_13N_2O_4$ : 468.0411. Found: 468.0410. = + 103.8° (c = 0.42, CHCl<sub>3</sub>).

(11aS) -7-Phenyl-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4] benzodiazepin-5-one (136, AG/129)

Cd/Pb (0.47 g) couple was added portion wise to a vigorously stirred solution of 135 (0.33 g, 0.7 mmol) in THF (5 mL) and of aq. ammonium acetate (1M, 5mL). The suspension was allowed to stir at room temperature for 2 hours, then poured into ethyl acetate (200 mL), dried with MgSO<sub>4</sub> and filtered. The filtrate was evaporated and the residue purified by flash column chromatography (ethyl acetate) to afford the title compound as colourless oil (0.19 g, 98%): <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) δ 2.0-2.12 (m, 2H); 2.29-2.37 (m, 2H); 3.53-3.63 (m, 1H); 3.76-3.92 (m, 2H); 7.36-7.79 (m, 8H); 8.28 (d, J = 2.2 Hz, 1H). <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>) δ 24.4; 29.8; 46.9; 53.8; 126.9; 127.3; 127.7; 128.0; 128.2; 128.8; 128.9; 129.1; 130.1; 130.5; 139.5; 145.0; 164.5; 165.1. IR (Nujol): cm<sup>-1</sup> 3000-2800, 1620, 1455, 1377, 1239, 1239, 1014, 990, 761,728, 697. HRMS Calculated for C<sub>18</sub>H<sub>1</sub>6N<sub>2</sub>O: 276.1261. Found: 276.1262. [al<sup>25</sup><sub>p</sub> = + 131.4° (c = 0.19, CHCl3).

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Example 3(i): Synthesis of the 7-(4'-Methoxyphenyl)-PBD (138, AG/135)

20 (11S,11aS)-7-(4'-Methoxy)phenyl-11-hydroxy-10-N-(2'',2'',2''trichloroethoxycarbonyl)-1,2,3,10,11a-hexahydro-5H-pyrrolo[2,1c]-[1,4]benzodiazepin-5-one (137)

134 (0.5 g, 1.0 mmol), 4-methoxybenzeneboronic acid (0.19 g, 1.2

mmol),  $Pd(PPh_3)_4$  (15 mg) and anhydrous  $Na_2CO_3$  (0.16 g, 1.48 mmol) were heated at reflux, over night, in a mixture of distilled benzene (20 mL), ethanol (2 mL) and water (2 mL). The reaction mixture was diluted with ethyl acetate (20 mL) and washed with 5 water (2 x 20 mL). The organic phase was dried over MgSO₄ and evaporated to yield a crude yellow oil. Purification by flash chromatography (ethyl acetate/petroleum ether 50/50) afforded the pure compound (0.34 q, 71%):  ${}^{1}H$  NMR (CDCl<sub>3</sub>)  $\delta$  1.96-2.16 (m, 4H); 3.54-3.63 (m, 2H); 3.71-3.79 (m, 1H); 3.85 (s, 3H); 4.18 (d, J =10 4.8 Hz, 1H); 4.29 (d, J = 12.1 Hz, 1H); 5.20 (d, J = 12.1 Hz, 1H); 5.66-5.72 (dd, J = 4.5, J = 9.8 Hz, 1H); 6.97 (d, J = 8.8Hz, 2H); 7.37 (d, J = 8.2 Hz, 1H); 7.57 (d, J = 8.8 Hz, 2H); 7.64(dd, J = 2.4, J = 8.2 Hz, 1H); 7.97 (d, J = 2 Hz, 1H). <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  23.0; 28.7; 46.4; 55.4; 59.6; 75.1; 86.1; 94.9; 114.3; 126.8; 129.1; 130.6; 131.7; 132.0; 132.2; 132.3; 15 133.5; 140.7; 154.5; 159.6; 166.9. IR (Nujol): cm<sup>-1</sup> 3000-2800, 1740, 1620, 1462, 1378, 1247, 1082, 816, 721. MS: m/e (relative intensity) 498 (M<sup>+</sup>, 15), 350 (20), 321 (15), 252 (100), 196 (22), 182 (5), 126 (7), 70 (28). HRMS Calculated for  $C_{22}H_21Cl_3N_2O_5$ : 498.0515. Found: 498.0513. [a]  $^{25}_{D} = +149.4$ . 20  $(0.25, CHCl_3)$ 

(11aS)-7-(4'-Methoxyphenyl)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c]-[1,4]benzodiazepin-5-one (138, AG/135)

Cd/Pb couple (0.51 g) was added portion wise to a, vigorously

stirred, solution of 137 (0.34 g, 0.76 mmol) in THF(5 mL) and

aq. ammonium acetate (1M, 5 mL). The suspension was allowed to

stir at room temperature for 2 hours, then poured into ethyl

acetate (200 mL), dried over MgSO4 and filtered. The organic solution was evaporated and the residue purified by flash column chromatography (ethyl acetate), to afford the title compound as colourless oil (0.1 g, 70%):  $^{1}$ H NMR (CDCl<sub>3</sub>, DMSO- $d_6$ )  $\delta$  2.1 (m, 2H); 2.3-2.4 (m, 2H); 3.5-3.62 (m, 1H); 3.85 (m, 5H); 7.0 (d, J=5 8.8 Hz, 2H); 7.36 (d, J = 8.3 Hz, 2H); 7.6 (d, J = 8.8 Hz, 2H); 7.72 (dd, J = 2.2, J = 8.2 Hz 1H); 7.8 (d, J = 4.4 Hz, 1H,); 8.2 (d, J = 2.2 Hz, 1H). <sup>13</sup>C NMR (270 MHz, CDCl<sub>3</sub>, DMSO- $d_6$ )  $\delta$  24.1; 29.5; 46.7; 53.6; 55.3; 77.3; 114.1; 114.3; 127.4; 127.6; 127.8; 128.0; 129.3; 131.9; 138.7; 144.3; 159.4; 164.2; 164.8. IR 10  $(Nujol): cm^{-1} 3000-2800, 1662, 1607, 1491, 1454, 1245, 1069, 823,$ 759. MS: m/e (relative intensity) 306 (M<sup>+</sup>, 100), 277 (15), 237 (10), 182 (12), 153 (10), 132 (5), 70 (10). HRMS Calculated for  $C_{19}H_18N_2O_2$ : 306.1367. Found: 306.1365. [a]  $^{25}_{p} = +773.1^{\circ}$  (c = 0.11, CH<sub>3</sub>OH). 15

Example 3(j): Synthesis of the 7-(3'-Nitrophenyl)-PBD (140, AG/150)

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(11S,11aS)-7-(3'-Nitro)phenyl-11-hydroxy-10-N-(2'',2'',2''-trichloroethoxycarbonyl)-1,2,3,10,11a-hexahydro-5H-pyrrolo[2,1-c]-[1,4]benzodiazepin-5-one (139)

134(0.5 g, 1.0 mmol), 3-nitrobenzeneboronic acid (0.2 g, 1.2

mmol),  $Pd(PPh_3)_4$  (25 mg) and anhydrous  $Na_2CO_3$  (0.16 g, 1.48 mmol)

were heated at reflux, over night, in a mixture of distilled benzene (20 mL), ethanol (2 mL) and water (2 mL). mixture was diluted with ethyl acetate (20 ml) and washed with water (2 x 20 ml). The organic phase was dried over MgSO<sub>4</sub> and evaporated to yield a crude yellow oil. Purification by flash chromatography (ethyl acetate/petroleum ether 50/50) afforded the pure compound (0.45 g, 90%):  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  2.0-2.2 (m, 4H); 3.6 (m, 2H); 3.76 (m, 1H); 4.31 (d, J = 12 Hz, 1H); 5.19 (d, J = 12 Hz, 1H); 5.76 (d, J = 10 Hz, 1H); 7.5-8.5 (m, 8H). $^{13}\text{C}$  NMR (68.7 MHz, CDCl<sub>3</sub>)  $\delta$  22.9, 28.7, 46.4, 59.7, 75.0, 86.0, 10 94.8, 121.7, 122.6, 127.5, 129.4, 129.9, 131.2, 132.0, 132.8, 133.9, 138.3, 140.7, 148.6, 154.1, 166.3. IR (Nujol): cm<sup>-1</sup> 3000-2800, 1721, 1626, 1530, 1455, 1349, 1062, 821, 759. MS: m/e (relative intensity) 513  $(M^{+})$ , 336 (55), 321 (100), 292 (15), 267 (54), 221 (16), 197 (18), 164 (15), 70 (22). HRMS Calculated 15 for  $C_{21}H_18C_13N_3O_6$ : 515.0233. Found: 515.0235. [a]  $^{25}_{p} = + 129.6$ (C = 0.1, CH<sub>3</sub>OH).

(11aS)-7-(3'-Nitrophenyl)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (140, AG-150)

A solution of TBAF in THF (1M solution, 7.6 mL, 7.6 mmol) was added to a solution 139 (0.39 g, 0.8 mmol) in of THF (20 mL) and the reaction mixture allowed to stir for 2 hours at room temperature. The solution was diluted with ethyl acetate (50 mL) and washed with water (3 x 50 mL) to remove excess TBAF. The organic phase was dried over MgSO<sub>4</sub> and evaporated to dryness. The residue was purified by flash column chromatography (CHCl<sub>3</sub>), to afford the title compound as a colourless oil (0.15 g, 63%):

<sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  1.8-2.2 (m, 3H); 3.5-4.0 (m, 3H); 7.3-8.5 (m, 7H). IR (Nujol): cm<sup>-1</sup> 3000-2850, 1624, 1527, 1466, 1349, 1244, 757, 740. MS: m/e (relative intensity) 321 (M<sup>+</sup>, 100), 292 (8), 265 (5), 224 (5), 197 (7), 151 (5), 70 (5). HRMS Calculated for C<sub>18</sub>H<sub>1</sub>5N<sub>3</sub>O<sub>3</sub>: 321.1115. Found: 321.1113. [a]<sup>25</sup><sub>D</sub> = + 129.6 (c = 0.1, CH<sub>3</sub>OH).

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(141)

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# Example 3(k): 8-Benzyloxy-7,9-dimethoxy-1,2,3,11a tetrahydropyrrolo[2,1-c][1,4]benzodiazepin-5-one (143, DRH-105) (see Figure 19

N-(4-Benzyloxy-3,5-dimethoxy-2[trichloroethyloxycarbonylamino]benzoyl)pyrrolidine-2-methanol

A solution of 2,2,2-trichloroethyl chloroformate (1.08 g, 4.8 mmol) in distilled dichloromethane (10 mL) was added dropwise over 0.5 h to a solution of anhydrous pyridine (0.80 g, 10.1 mmol) and 126 (Example 3(g))(1.95 g, 5.1 mmol) in distilled dichloromethane (20 mL) at 0° C. After 1 h the reaction mixture was diluted with anhydrous DCM (100 mL) and washed with 1N HCl (2 x 100 mL), H<sub>2</sub>O (100 mL), brine (100 mL) and dried over anhydrous MgSO<sub>4</sub>. Evaporation of the solvent yielded a brown oil which was purified by flash column chromatography (silica gel, EtOAc) to afford the product as a yellow glass (2.65 g, 4.7 mmol, 94%); <sup>1</sup>H

NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  1.6-2.2 (m, 4H), 3.3-3.6 (m, 2H), 3.6-3.9 (m, 2H), 3.8 (s, 3H), 3.9 (s, 3H), 4.2-4.3 (m, 1H), 4.8 (s, 2H), 5.1 (s, 2H), 6.6 (s, 1H), 7.2 (br s, 1H), 7.3-7.5 (m, 5H);  $^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  171.5, 153.1, 142.0, 137.023, 128.3, 128.3, 128.2, 120.1, 105.3, 95.4, 75.3, 74.6, 66.5, 61.4, 61.3, 56.3, 50.7, 28.7, 24.6.

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(11S,11aS)-8-benzyloxy-7,9-dimethoxy-11-hydroxy-10-N-(2',2',2'-trichloroethoxylcarbonyl)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4] benzodiazepin-5-one (142)

Anhydrous DMSO (0.97 q, 12.5 mmol) in dry DCM (10 mL) was added 10 dropwise over 30 minutes to a stirred solution of oxalyl chloride (3.08 mL of a 2N solution in DCM, 6.2 mmol) in dry DCM (10mL) under a nitrogen atmosphere at -45° C. After stirring for 15 mins, the substrate (2.46 q, 4.38 mmol) in dry DCM (25 mL) was 15 added dropwise over 45 minutes to the reaction mixture, which was then stirred for a further 45 minutes at -45° C. TEA (1.77 g; 17.5 mmol) was added dropwise to the mixture over 0.5 h and stirred for a further 15 minutes. The reaction mixture was left to warm to room temperature, diluted with H2O (100 mL) and the phases allowed to separate. The organic phase was washed with 1N 20 HCl (2 x 50 mL), water (2 x 50 mL), brine (2 x 50 mL) and dried over MgSO<sub>4</sub>. The solvent was evaporated to afford the product as an off-white glass (3.92 g, 11.7 mmol; 97 %);  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  2.01-2.17 (m, 4H), 3.44-3.77 (m, 2H), 3.87-3.90 (m, 1H), 3.88 (s, 3H), 3.91 (s, 3H), 4.68 (dd, 2H), 5.01 (s, 2H), 5.62 (d, 25 1H), 7.08 (s, 1H), 7.27-7.48 (m, 5H);  $^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$ 166.7, 155.2, 153.6, 150.5, 143.6, 137.1, 129.8, 129.3, 128.4,

128.3, 128.2, 128.1, 121.8, 106.5, 106.3, 94.7, 86.2, 85.9, 75.6, 75.4, 75.2, 75.0, 61.8, 61.5, 60.2, 59.870, 56.1, 56.0, 46.5, 46.3, 45.8, 28.7, 28.6, 23.0.

8-Benzyloxy-7,9-dimethoxy-1,2,3,11a-tetrahydropyrrolo[2,1-c][1,4]benzodiazepin-5-one (143)

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10% Cd/Pb couple (1.2 g; 10 mmol Cd) was added to a rapidly stirring solution of 142 (1.08 g; 1.9 mmol) in a mixture of THF (15 mL) and 1N NH<sub>4</sub>OAc (15 mL). After 3.5 h, TLC revealed that reaction was still incomplete and more 10% Cd/Pb couple (500 mg) was added. After a further 1 h the reaction mixture was diluted with EtOAc (150 mL). The solution was dried over anhydrous MgSO<sub>4</sub> and the solids were filtered and rinsed with EtOAc (50 mL). Removal of excess solvent yielded the product as a yellow glass (0.48 g, 1.3 mmol, 68%). H<sup>1</sup> NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  7.73 (d, 1H, J = 4.4 Hz), 7.36 (s, 2H), 7.31 (s, 2H), 7.11 (s, 1H), 7.08 (s, 1H), 5.12 (br s, 2H), 3.98-3.42 (m, 9H), 2.38-2.29 (m, 2H), 2.23-1.83 (m, 2H).

# Example 3(1): Synthesis of the C8-NH2 PBD (157, AG/149) (see Figure 20)

4-Nitro-2-(2',2',2'-trichloroethoxycarbonylamino)benzoic acid (145)

A solution of 2,2,2-trichloroethylchloroformate (Troc-Cl) (1.66

mL, 12.1 mmol) in dry dichloromethane (25 mL) was added drop wise to a solution of 4-nitroanthranilic acid 144 (2 g, 11 mmol) and pyridine (1.78 mL, 22 mmol) in dichloromethane (25 ml) at 0°C. The solution was allowed to stir at 25°C for 5 hours. The reaction mixture was washed with dilute HCl (1N, 2 x 50 mL), water (1 x 50 mL), brine (1 x 2 5 mL) and dried over MgSO<sub>4</sub>. Removal of excess solvent by rotary evaporation under reduced pressure afforded the crude product which was used in the subsequent reaction without further purification.

N-[4-nitro-(2',2',2'-trichloroethoxycarbonylamino) benzoyl]

pyrrolidine-2-methanol (146)

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Oxalyl chloride (1 mL, 12.1 mmol) and a catalytic amount of dry DMF were added to a suspension of the crude product from the previous reaction in of dry dichloromethane (50 mL) and the reaction mixture was allowed to stir at room temperature for 12 hours. The newly formed acid chloride was added drop wise, over 1 hour, to a solution of 2S-(+)-pyrrolidinemethanol (1.22 g, 12.1 mmol) and triethylamine (3.8 mL, 27.5 mmol) in dichloromethane (50 mL) at -20° C (CCl<sub>4</sub>-dry ice). The reaction mixture was stirred for a further hour at -20° C and was then allowed to warm to room temperature. The reaction mixture was washed with dilute HCl (1N, 2 x 50 mL), water (50 mL) and brine (50 mL), dried over MgSO<sub>4</sub> and evaporated under reduced pressure. The residue was purified by flash chromatography (EtoAc/petroleum ether 50/50), removal of excess eluent afforded of a yellow oil (1.34 g, 30%, over two steps): <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) & 1.7 - 2.3 (m, 4H);

3.45 (m, 2H); 3.71 (dd, J = 5.5, J = 11, 1H); 4.06 (m, 2H); 4.43

(bs, 1H); 4.85 (d, J = 13, 1H); 4.89 (d, J = 13 Hz, 1H); 7.56 (d, J = 8.4 Hz, 1H); 7.96 (dd, J = 2.2, J = 8.4 Hz, 1H); 8.94 (d, J = 2.2 Hz, 1H); 9.2 (bs, 1H). <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  24.9; 27.9; 50.8; 60.5; 64.3; 74.6; 94.9; 115.9; 117.9; 128.6; 130.5; 136.9; 149.0; 151.8; 167.7. MS: m/e (relative intensity) 441 ([M+1], 1), 291 (10), 260 (12), 191 (30), 164 (15), 154 (8), 113 (20), 77 (20), 70 (100). HRMS Calculated for  $C_{15}H_{16}C_{13}N_{3}O_{6}$ : 439.0104. Found: 439.0105. [a]<sup>25</sup>  $_{D} = -110.6$ ° (C = 0.13, CHCl3).

# N-[4-amino(2',2',2'-trichloroethoxycarbonylamino)benzoyl] pyrrolidine-2-methanol (147)

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A solution of 146 (1 g, 2.3 mmol) and  $SnCl_2$  2H<sub>2</sub>O (2.56 g, 11.4 mmol) in methanol (20 mL) was heated at reflux for 6 hours (the reaction was monitored by TLC (3% methanol, ethyl acetate). reaction mixture was reduced to 1/3 of it's original volume and the pH adjusted to 8-9 with satd. aqueous NaHCO3. Ethyl acetate 15 (100 mL) was added and the mixture was vigorously stirred for 12 hours, then filtered through Celite to remove tin salts. organic phase was dried over MgSO4 and evaporated to afford the product as a yellow oil (0.94 g, 97%) which was used in the next reaction without further purification:  $^{1}\text{H}$  NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$ 20 1.6 - 1.8 (m, 2H); 1.9 (m, 1H); 2.17 (m, 1H); 3.48 - 3.58 (m, 1H); 3.62 - 3.72 (m, 2H); 3.84 (m, 1H); 4.44 (m, 1H); 4.77 (d, J= 12.1 Hz , 1H); 4.83 (d, J = 12.1 Hz, 1H); 6.32 (dd, J = 2.2, J= 8.43 Hz, 1H; 7.18 (d, J = 8.43 Hz, 1H); 7.52 (d, J = 2.2 Hz,1H); 9.62 (bs, 1H).  $^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  21.1; 25.2; 25 28.2; 51.9; 60.9; 66.5; 74.3; 95.3; 105.5; 108.3; 112.6; 130.1; 138.9; 149.7; 151.8; 171.5. IR (Nujol): cm<sup>-1</sup> 3346, 3000-2800,

1738, 1620, 1463, 1196, 1046, 963, 820 760. MS: m/e (relative intensity) 409 ([M-1], 15), 309 (20), 179 (25), 161 (100), 134 (8), 113 (25), 77 (35), 70 (85). HRMS Calculated for  $C_{15}H_{18}Cl_{3}N_{3}O_{4}$ : 409.0362. Found: 409.0363. [a]  $^{25}_{D}$  = -60.1° (c = 0.3, CHCl<sub>3</sub>).

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N-[4-(Fmoc)amino(2',2',2'-trichloroethoxycarbonylamino)benzoyl]
pyrrolidine-2-methanol (148)

An aqueous solution of NaHCO<sub>3</sub> (0.6 g, 5.67 mmol, in 20 mL of  $H_2O$ ) was added to a solution of 147 (0.94 g, 2.3 mmol) in THF (20 mL). The reaction mixture was cooled to 0°C and Fmoc-Cl (0.65 g, 2.5 10 mmol) was added in small portions. After addition the reaction mixture was allowed to stir for 2 hours at room temperature. (TLC: ethyl acetate /petroleum ether 50/50). The reaction mixture was acidified with dilute HCl (1N) and extracted with ethyl acetate (2 x 20 mL). The organic phase was dried (MgSO<sub>4</sub>) 15 and evaporated and the resulting yellow oil thus obtained was purified by flash chromatography to afford the product (1.03 g, 72%):  $^{1}\text{H}$  NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  1.68 (m, 2H); 1.84 (m, 1H); 2.11 (m, 1H); 3.48 (m, 2H); 3.71 (m, 1H); 3.87 (m, 1H); 4.19 (t, <math>J =6.8 Hz, 1H); 4.40 (m, 2H); 4.45 (d, J = 6.78 Hz, 2H); 4.73 (d, J20 = 12.1, 1H); 4.78 (d, J = 12.1 Hz, 1H); 7.2 - 7.8 (m, 11H); 8.04 (bs, 1H).  $^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  25.1; 28.1; 46.8; 51.6; 60.8; 65.7; 67.1; 74.3; 95.2; 109.9; 112.3; 118.3; 120.0; 124.9; 127.1; 127.8; 129.3; 137.5; 140.9; 141.2; 143.6; 151.8; 153.2; 170.3. IR (Nujol): cm<sup>-1</sup> 3301, 3000-2800, 1738, 1599, 1525, 1451, 25 1224, 1056, 985, 758, 740, 667. MS: m/e (relative intensity) 632  $(M^{+})$ , 409 (15), 309 (20), 179 (25), 161 (100), 134 (8), 113

(25), 77 (35), 70 (85).  $[a]_{D}^{25} = -70.3$  (c = 0.25, CHCl3).

(11S, 11aS) -8- (Fmoc) amino-11-hydroxy-10-N- (2', 2', 2'-trichloroethoxycarbonyl) -1, 2, 3, 10, 11, 11a-hexahydro-5H-pyrrolo[2, 1-c][1,4] benzodiazepin-5-one (149)

A solution of DMSO (0.31 ml, 4.4 mmol) in of dry dichloromethane 5 (10 mL) was slowly added (over 30 minutes) to a solution of oxalyl chloride (2.2 mmol) in dry dichloromethane (11.1 mL) at 45° C. The mixture was allowed to stir for 15 minutes followed by the addition of a solution of 148 (1 g, 1.58 mmol) in of dry dichloromethane (15 ml), keeping the temperature below -40° C. 10 After further 60 minutes at -45° C, a solution of triethylamine (0.88 ml 6.32 mmol) in dichloromethane (6 mL) was added and the reaction mixture allowed to warm to room temperature. reaction mixture was washed with water (50 mL), dilute HCl (1N, 50 mL) and brine (50 mL). Evaporation of solvent afforded the 15 crude product which was purified by flash chromatography (ethyl acetate/petroleum ether 50/50). Removal of excess eluent furnished the product as a pale yellow oil (0.81 g, 82%): 1H NMR  $(CDCl_3)$   $\delta$  1.96 - 2.16 (m, 4H); 3.47 - 3.56 (m, 3H); 3.6 (m, 1H); 4.1 - 4.28 (m, 3H); 4.46 (d, J = 6.15 Hz, 2H); 5.01 (d, J = 12.120 Hz, 1H); 5.64 (d, J = 12.1 Hz 1H); 7.22 - 7.76 (m, 11H). <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  22.9; 28.7; 46.4; 46.9; 59.9; 67.0; 75.1; 86.0; 94.8; 117.7; 119.6; 120.1; 124.9; 127.9; 129.8; 134.9; 140.8; 141.3; 143.5; 153.0; 154.1; 166.7. IR (Nujol): cm<sup>-1</sup> 3282, 3000-2800, 1713, 1610, 1533, 1451, 1220, 1058, 908, 735, 647 MS: 25 m/e (relative intensity) 631 ([M+2], 1), 196 (5), 178 (100), 152

(5), 89 (7), 70 (10). HRMS Calculated for  $C_{30}H_{2}6Cl_{3}N_{3}O_{6}$ :

629.0887. Found: 629.0887. [a] $^{25}_{D}$  = + 58.7° (c = 0.5, CHCl3).

(11S,11aS)-8-amino-11-hydroxy-10-N-(2',2',2'-trichloroethoxycarbonyl)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (150)

- The protected carbinolamine 149 (0.8 g, 1.3 mmol) was added to a 5 5% solution of piperidine in CH<sub>3</sub>CN (12 mL, 5 eq. of piperidine). The mixture was allowed to stir for 12 hours, extracted with water (2 x 50 mL) and the organic phase was evaporated under reduced pressure to yield a pale yellow oil (0.24 g, 50%): 1H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  1.9 - 2.2 (m, 4H); 3.45 - 3.7 (m, 3H); 4.26 10 (d, J = 12.1 Hz, 1H); 4.55 (m, 3H); 5.18 (d, J = 12.1 Hz, 1H); 5.61 (d, J = 10.3 Hz, 1H); 6.61 (s, 1H); 6.69 (d, J = 7.3 Hz, 1H); 7.56 (d, J = 8.2 Hz, 1H). <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>)  $\delta$  23.0; 28.7; 46.3; 59.8; 74.9; 95.1; 114.8; 116.5; 130.4; 135.3; 154.4; 167.3. IR (Nujol):  $cm^{-1}$  3340, 3224, 3000-2800, 1714, 1602, 1460, 15 1311, 1208, 1141, 1061, 826, 759, 665. MS: m/e (relative intensity) 407 (M+, 40), 381 (5), 340 (10), 309 (25), 161 (100), 134 (15), 105 (15), 70 (80). HRMS Calculated for  $C_{15}H_16C_13N_3O_4$ : 407.0206. Found: 407.0206. [a]  $^{25}_{p} = +47.8^{\circ}$  (c = 0.5, CHCl3).
- 20 Synthesis of (11aS)-8-amino-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (151)

  Cd/Pb couple (5 eq, 0.34 g) was added portion wise to a vigorously stirred solution of 150 (0.2 g, 0.5 mmol) in THF (10 mL) and aqueous ammonium acetate (10 mL). Stirring was allowed to continue for a further 2 hours at room temperature and the

reaction mixture was poured into ethyl acetate (100 mL). The organic phase was dried over MgSO<sub>4</sub>, filtered and evaporated to yield the crude product which was subjected to flash chromatography (silica gel, 5% MeOH, 95% CHCl<sub>3</sub>). Removal of excess eluent afforded the product as a white solid (26 mg, 53% yield):  $^{1}$ H NMR (270 MHz, CDCl<sub>3</sub>, CD<sub>3</sub>OD)  $\delta$  1.6 - 2.2 (m, 4H); 3.2 - 3.4 (m, 2H); 3.5 (m, 1H); 5.0 (m, 2H); 6.05 (m, 1H); 6.25 (m, 1H); 7.43 (m, 1H), 7.75 (m, 1H). IR (Nujol): cm-1 3304, 3000-2800, 1613, 1457, 1377, 1244, 1202, 1122, 1072, 825, 759, 721. MS: m/e (relative intensity) 215 (M<sup>+</sup>, 100), 186 (15), 178 (10), 146 (10), 119 (25), 91 (15),70 (30), 65 (5). HRMS Calculated for C<sub>12</sub>H<sub>1</sub>3N<sub>3</sub>O: 215.1058. Found: 215.1059. [a]  $^{25}$  = + 163.3° (c = 0.2, CHCl<sub>3</sub>).

### Example 4: Synthesis of the C8-Amines

Synthesis of 3-(11-Hydroxy-5-oxo-10-(2,2,2-trichloroethyloxocarbonylamino)-(11aS)-2,3,5,10,11,11a-hexahydro
1H-benzo[e]pyrrolo[2,1-a][1,4]diazepin-8-yloxy-2
propenylpropanoate (159) (see Figure 21)

#### Nitro Di-acid (153)

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20 14.63 g of (4-carboxy-2-methoxy-5hydroxy-phenoxy) propanoic acid

152 (61 mmol) was added portionwise to 70% nitric acid (100 mL)

stirred at 0°C. The reaction was stirred for 1 h at 0°C then

allowed to return to rt. The reaction mixture was then poured onto ice and allowed to stir for 18 h. The solids were then collected by filtration and washed with water. The aqueous layer was then extracted with ethyl acetate (3 x 150 mL). were then washed with water and brine and dried with sodium 5 sulphate. The solvent was then removed in vacuo to give 153 as a yellow solid, yield = 14.01 g (83%) mp 141 °C. $^{1}$ H NMR (CDCl $_{3}$ ):  $\delta$ 8.51 (bs, 2H, COOH), 7.57 (s, 1H, CHCNO<sub>2</sub>), 7.15 (s, 1H, CH<sub>3</sub>OCCH), 4.35 (t, 2H, J = 6.41 Hz,  $CH_2CH_2O$ ), 3.99 (s, 1H,  $OCH_3$ ), 2.86 (t, 2H, J = 6.41 Hz,  $CH_2CH_2O$ ). <sup>13</sup>C-NMR (CDCl<sub>3</sub>):  $\delta$  33.93 (CH<sub>2</sub>CH<sub>2</sub>O), 10 56.42 (OCH<sub>3</sub>), 65.20 (CH<sub>2</sub>CH<sub>2</sub>O), 108.27 (NO<sub>2</sub>CCH), 111.26 (CH<sub>3</sub>OCCH), 122.50 (CCOOH), 141.14 (CNO<sub>2</sub>), 149.21 (CH<sub>2</sub>CH<sub>2</sub>OC), 152.40 (CH<sub>3</sub>OC), 166.93 (arom. COOH), 172.24 (aliph. COOH). IR (Nujol) v 2860, 2620, 1740, 1720, 1590, 1540, 1480, 1390, 1350, 1290, 1230, 1250, 1200, 1060 cm<sup>-1</sup>. EIMS m/e (relative intensity) : 286 (M<sup>+</sup>·, 20), 15 241 (10), 213 (100), 169 (20), 152 (5), 111 (20), 96 (5), 79 (5), 73 (15), 55 (10). HRMS Calcd. for  $C_{11}H_{14}NO_8 = 285.0511$  found = 285.0538.

2-Propene 3-(4-carboxy-2-methoxy-5-nitrophenoxy) propanoate (154)

20 A mixture of 3-(4-carboxy-2-methoxy-5-nitrophenoxy) propanoic acid
(153) (20 g, 74.3 mmol) and p-toluene sulphonic acid monohydrate
(2.3 g, 7.4 mmol) in allyl alcohol (240 mL, 3.5 mol) was refluxed
for 7 h then allowed to cool. The allyl alcohol was then removed
in vacuo, and the residue triturated with dilute HCl acid and
collected by filtration. This solid was taken up in EtOAc, and
the resulting solution washed with water and brine and dried over
sodium sulphate. Evaporation in vacuo afforded 154 as a white

solid (19.27 g, 84%): mp 128-130 °C;  $^{1}$ H-NMR (CDCl<sub>3</sub>):  $\delta$  2.92 (t, 2H, J = 6.35 Hz); 3.94 (s, 3H); 4.38 (t, 2H, J = 6.41 Hz); 4.65 (d, 2H, J = 5.61 Hz); 5.27 (dd, 1H,  $J_1$  = 1.28 Hz,  $J_2$  = 19.42 Hz); 5.33 (dd, 1H,  $J_1$  = 1.28 Hz,  $J_2$  = 17.04 Hz); 5.92 (m, 1H); 7.15 (s, 1H); 7.45 (s, 1H);  $^{13}$ C NMR (67.8 MHz, CDCl<sub>3</sub>):  $\delta$  34.1, 56.5, 65.0, 65.4, 108.5, 111.3, 118.3, 122.9, 131.8, 141.1, 149.1, 152.6, 167.1, 170.0; IR (Nujol);  $\nu$  1730, 1630, 1550, 1430, 1390, 1290, 1230, 1190, 1170, 1070, 1030, 1010 cm<sup>-1</sup>; MS (EI) m/z (relative intensity): 325 (M<sup>+</sup>, 19), 251 (3), 213 (2), 196 (3), 211 (3), 113 (19), 91 (4), 71 (9), 55 (6); HRMS: calcd. for  $C_{14}H_{15}NO_{8}$  325.0798, found 232.0773.

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# Prop-2-enyl 4-(N-2S-Diethylthiomethylpyrrolidinecarboxy)-2-methoxy-5-nitrophenoxy) propanoate (155)

2-Propene 3-(4-carboxy-2-methoxy-5-nitrophenyloxy)propanoate

(154): 5 g, 15.34 mmol), oxalyl chloride (2 mL, 23 mmol) and 5 15 drops of DMF were stirred in dry THF (100 mL) for 18 h. solvent was then removed in vacuo and the residue dissolved in dry THF (50 mL). This was added dropwise to a vigorously stirred mixture of (2s)-pyrrolidone-2-caroxaldehyde diethyl thioacetate(3.15 g, 15.34 mmol) and triethylamine (1.86 g, 18.41 20 mmol). The stirring was continued for 18 h. The solvent was then removed in vacuo and the product purified by flash chromatography eluting with ethyl acetate to give 155 (7.48g, 95%) as a yellow oil.  $^{1}$ H NMR (CDCl<sub>3</sub>):  $\delta$  7.74 (s, 1H, OCCHC), 6.83 (s, 1H, MeOCCHC), 5.98-5.86 (m, 1H,  $CH_2CHCH_2$ , 5.33 (d, 1H, J =25 26.56 Hz,  $OCH_2CHCH_2$ ), 5.28 (d, 1H, J = 20.24 Hz,  $OCH_2CHCH_2$ ), 4.88 (d, 1H, J = 3.85 Hz, NCHCH),  $4.74-4.65 \text{ (m, 2H, OCH_2CHCH_2)}$  4.42

(t, 2H, J = 7.69 Hz, CH<sub>2</sub>CH<sub>2</sub>OC), 3.94 (s, 3H, OCH<sub>3</sub>), 3.29-3.21 (m, 2H, NCH<sub>2</sub>), 2.96 (p, 2H, J = 3.12 Hz, CH<sub>2</sub>CH<sub>2</sub>O), 2.87-2.67 (m, 4H, SCH<sub>2</sub>CH<sub>3</sub>), 2.32-1.78 (m, 4H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>)1.38-1.31 (m, 6H, SCH<sub>2</sub>CH<sub>3</sub>).

1<sup>3</sup>C-NMR (CDCl<sub>3</sub>): δ 15.00, 15.13 (SCH<sub>2</sub>CH<sub>3</sub>), 24.63 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>),

5 26.28, 26.59, 27.22 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 34.13 (CH<sub>2</sub>CH<sub>2</sub>O), 50.19 (NCH<sub>2</sub>),

52.80 (NCHCH), 56.60 (OCH<sub>3</sub>), 61.08 (NCH), 65.13 (CH<sub>2</sub>CH<sub>2</sub>O), 65.64 (OCH<sub>2</sub>CHCH<sub>2</sub>), 108.70 (arom. CH), 109.47 (arom. CH), 118.55 (OCH<sub>2</sub>CHCH<sub>2</sub>), 128.58 (CCON), 131.73 (OCH<sub>2</sub>CHCH<sub>2</sub>), 137.17 (CNO<sub>2</sub>),

147.98 (CH<sub>2</sub>CH<sub>2</sub>OC), 154.57 (COCH<sub>3</sub>), 166.61 (CON), 170.14 (COO).

10 IR (Nujol) ν= 3550-2720, 3000, 2630, 2200, 1740, 1640, 1580, 1530, 1340, 1280, 1220, 1180, 1050 cm<sup>-1</sup>. MS (EI): m/e (relative intensity): 527 (M<sup>+</sup>, 1), 377 (10), 310 (12), 309 (72), 308 (94), 268 (20), 142 (4). HRMS calcd. for C<sub>24</sub>H<sub>35</sub>O<sub>7</sub>N<sub>2</sub>S<sub>2</sub> = 527.1875, found = 527.1885.

5-Amino-3-(4-(2-diethylthiomethyl-(2S)-perhydro-1-15 pyrroloylcarbonyl) -2-methoxyphenyloxy) 2-propenylpropanoate (156) 8 (7.21 g, 14.05 mmol) and Tin(II) chloride (15.85 g, 76 mmol) was refluxed for 40 min in ethyl acetate (100 mL) then allowed to cool. The solvent was then removed in vacuo and the residue was triturated with saturated bicarbonate solution at 0°C. 20 (50 mL) was added and the reaction stirred overnight. reaction mixture was then filtered through Celite and the filter cake washed with ethyl acetate. The combined organics were then washed with water and brine, dried with sodium sulphate and the solvent removed in vacuo. The product was purified using flash 25 chromatography eluting with 5% MeOH in dichloromethane to give a yellow oil, yield = 5.87g (86%). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  6.82 (s, 1H,

arom. CH), 6.28 (s, 1H, arom.CH), 5.99-5.85 (m, 1H, OCH<sub>2</sub>CHCH<sub>2</sub>), 5.31 (dd, 1H, J = 1.28 Hz, 27.66 Hz, OCH<sub>2</sub>CHCH<sub>2</sub>), 5.26 (dd, 1H, J = 1.28 Hz, 20.70 Hz,  $OCH_2CHCH_2$ ), 4.71-4.62 (m, 5H, including doublet at 4.62, 2H, J = 5.49 Hz,  $NH_2 + NCHGH$ ,  $OCH_2CHCH_2$ ), 4.27  $(t, 2H, J = 6.59 \text{ Hz}, CH_2CH_2O), 3.92, (m, 1H, NCH), 3.74 (s, 3H,$ 5  $OCH_3$ ), 3.66-3.57 (m, 2H,  $NCH_2$ ) 2.89 (t, 2H, J = 6.6 Hz,  $CH_2CH_2O$ ), 2.83-2.64 (m, 4H, SCH<sub>2</sub>CH<sub>3</sub>), 2.28-1.80 (m, 4H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.25 (m, 6H,  $SCH_2CH_3$ ); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  14.20 (SCH<sub>2</sub>CH<sub>3</sub>), 26.55, 27.23  $(NCH_2CH_2CH_2)$ , 34.27  $(CH_2CH_2O)$ , 53.20 (NCHCH), 56.08  $(OCH_3)$ , 60.10 (NCH), 60.39  $(NCH_2)$ , 64.20  $(CH_2CH_2O)$ , 64.41  $(OCH_2CHCH_2)$ , 102.26 10 (arom. CH), 113.71 (arom. CH), 118.40 (OCH<sub>2</sub>CHCH<sub>2</sub>), 131.93  $(OCH_2CHCH_2)$ , 141.03  $(CNH_2)$ , 141.74  $(CH_2CH_2OC)$ , 154.56  $(COCH_3)$ , 169.69 (CON), 170.53 (COO). IR (neat liquid film) 3500-3000, 3460, 3400, 2970, 1740, 1650, 1535, 1470, 1345, 1290, 1225, 1190  $cm^{-1}$ ; MS (EI): m/e (relative intensity): 482 (M<sup>+</sup>·, 4), 347 (2), 15 278 (31), 137 (1), 70 (3); HRMS calcd. for  $C_{23}H_{34}O_5N_2S_2 =$ 482.1909, found = 482.1925.

3-(4-(2-Diethylthiomethyl-(2S)-perhydro-1-pyrrolylcarbonyl)-2-methoxy-5-(2,2,2-trichloroethyloxycarbonylamino)phenyloxy)2-propenylpropanoate (157)

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To a solution of 156 (5.67g, 11.74 mmol) in dichloromethane (200 mL) was added pyridine (2.02 mL, 23.48 mmol). To this was added dropwise at 0°C a solution of trichloroethyl chloroformate (1.616 mL, 11.74 mmol). The solution was stirred for a further 1 hour at 0°C. The organics were washed with 1 N HCl (3 X 100 mL), water (3 x 100 mL) brine (100 mL), dried over magnesium sulphate and the solvent removed in vacuo to give a brown oil (6.8g, 88%)

 $^{1}$ H NMR (CDCl<sub>3</sub>):  $\delta$  9.14 (bs, 1H, NH), 7.88 (bs, 1H, CHCNH), 6.93 (s, 1H, MeOCCHC), 5.99-5.86 (m, 1H, OCH<sub>2</sub>CHCH<sub>2</sub>), 5.31 (dt, 1H, J =1.47 Hz, 27.84 Hz  $OCH_2CHCH_2$ ), 5.25 (dt, 1H, J =1.29 Hz, 21.61 Hz,  $CH_2CHCH_2$ ), 4.89-4.77 (m, 4H, including doublet 1H, J = 1.28 Hz, CHCHSEt, NH,  $CH_2$ -TrOC), 4.62 (d, 2H, J =1.28 Hz,  $OCH_2$ CHCH<sub>2</sub>), 3.81 5  $(s, 3H, OCH_3)$ , 3.60  $(m, 2H, NCH_2)$ , 2.91 (d, 2H, J = 6.42 Hz, $CH_2CH_2O$ ), 2.84-2.61 (m, 4H,  $SCH_2CH_3$ ), 1.37-1.23 (m, 6H,  $SCH_2CH_3$ );  $^{13}$ C NMR (CDCl<sub>3</sub>):  $\delta$  170.33 (ester **C**O), 168.50 (**C**ON), 151.94 (O**C**O), 150.29 (COCH<sub>3</sub>), 144.52 (COCH<sub>2</sub>CH<sub>2</sub>), 131.93 (OCH<sub>2</sub>CHCH<sub>2</sub>), 131.35(CNH), 118.29 (OCH<sub>2</sub>CHCH<sub>2</sub>), 112.21 (arom. CH), 105.51 (arom. CH), 10 95.27 ( $CCl_3$ ), 76.24 ( $CH_2Troc$ ), 74.39 ( $CH_2Troc$ ), 65.42 ( $CH_2CH_2O$ ), 61.14 (NCH), 56.30 (OCH<sub>3</sub>), 53.00 (NCHCHSEt), 34.27 (CH<sub>2</sub>CH<sub>2</sub>O), 27.30, 26.71, 26.43, 25.24  $(NCH_2CH_2CH_2)$ , 15.27, 14.87, 14.18  $(SCH_2CH_3)$ . MS (EI): m/e (relative intensity): 658, 656  $(M^{+}, 1)$ , 508 (1), 373 (6), 305 (5), 304 (27), 192 (5), 70 (12). 15

3-(11-Hydroxy-5-oxo-10-(2,2,2-trichloroethyloxocarbonylamino)(11aS)-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[2,1a][1,4]diazepin-8-yloxy-2-propenylpropanoate (158)

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A solution of 157 (6.8g, 10.34 mmol) in acetonitrile/water (4:1, 200 mL) was treated with calcium carbonate (2.585g, 25.85 mmol) and mercuric(II) chloride (7.00g, 25.85 mmol) and the solution was stirred for 18 h. The reaction was then filtered through Celite and the filter pad washed with ethyl acetate. The organics were collected and washed with water (3 x 50 mL), brine (100 mL) and dried over magnesium sulphate. The solvent was removed in vacuo and the resulting product was purified by flash chromatography eluting with ethyl acetate to give the product as

a yellow oil (3.67 g, 64%)  $^{1}$ H NMR (CDCl<sub>3</sub>):  $\delta$  7.25 (arom. CH), 6.86 (s, 1H, arom. CH), 6.00-5.85 (m, 1H,  $CH_2CHCH_2$ ), 5.67 (d, 1H,  $J = 9.71 \text{ Hz}, \text{ TrOC-CH}_2) 5.37-5.20 (m, 3H, \text{TrOC-CH}_2 + \text{OCH}_2\text{CHCH}_2),$ 4.65 (d, 2H, J = 5.67 Hz,  $CH_2CHCH_2O$ ), 4.36-4.22 (m, 3H,  $CH_2CH_2O +$ NCHOH), 3.90 (s, 3H,  $OCH_3$ ), 3.72-3.47 (m, 3H,  $NCH + NCH_2$ ), 2.91 5 (t, J = 6.41 Hz,  $CH_2CH_2O$ ) 2.29-2.00 (m, 4H,  $NCH_2CH_2CH_2$ ) <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  170.33 (ester carbonyl CO), 166.17 (CON), 154.4 (OCO), 149.88 (COCH<sub>3</sub>), 148.93 (COCH<sub>2</sub>CH<sub>2</sub>), 131.86 (CH<sub>2</sub>CHCH<sub>2</sub>), 127.48 (arom. CN), 126.24 (CCON), 118.42 (OCH<sub>2</sub>CHCH<sub>2</sub>), 114.48 (arom. CH), 110.82 (arom. CH), 95.09 (CCl<sub>3</sub>), 86.42 (NCHOH), 74.96 (TrOC-CH<sub>2</sub>), 10  $65.47 \text{ (OCH}_2\text{CHCH}_2), 64.43 \text{ (CH}_2\text{CH}_2\text{O)}, 60.13 \text{ (NCH)}, 56.14 \text{ (OCH}_3),$  $46.44 \text{ (NCH}_2)$ ,  $34.26 \text{ (CH}_2\text{CH}_2\text{O)}$ ,  $28.64 \text{ (NCH}_2\text{CH}_2\text{CH}_2\text{),MS (EI) m/z}$ (relative intensity): = 552 ( $M^+$  10), 550 (10), 374 (2), 368 (5), 304 (15), 192 (8), 70 (24), 55(24). HRMS calcd. for  $C_{22}H_{25}N_2O_8Cl_3$ = 552.0651, found 3 peaks due to chlorine 552.0646, 550.676, 15 554.0617.

3-(11-Hydroxy-5-oxo-7-methoxy-10-(2,2,2-trichloroethyloxocarbonylamino)-(11aS)-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[2,1-a][1,4]diazepin-8-yloxypropanoic acid

20 (159)

A solution of 158 (3.5 g, 6.35 mmol) was dissolved in ethanol (100 mL). To this was added

Tetrakis(triphenylphospine)palladium(0) (350 mg, 0.303 mmol) and the solution refluxed for 30 minutes until the reaction was complete by TLC monitoring. The reaction was then allowed to cool and the filtered through Celite. The EtOH was then removed in vacuo to give the crude material as a yellow solid which was

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used directly in the next steps. <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ 7.22 (s,1H, OCCHCN), 7.01 (s, 1H, MeOCCHC), 6.27 (bs, COOH), 5.67 (d, 1H, J = 9.5 Hz, TrOC-CH<sub>2</sub>), 5.06 (d, 1H, J = 12.09 Hz, TrOC-CH<sub>2</sub>), 4.29-4.11 (m, 2H, CHOH), 3.85 (s, 3H, OCH<sub>3</sub>), 3.71 (t, 2H, J = 6.97 Hz, CH<sub>2</sub>CH<sub>2</sub>O), 3.51 (m, 1H, NCH), 2.80 (m, 2H, NCH<sub>2</sub>), 2.12-1.99 (m, 4H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.21 (t, 2H, J = 6.96 Hz, CH<sub>2</sub>CH<sub>2</sub>O) <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ = 174.27 (acid CH), 167.34 (CON), 154.20 (OCO), 149.78 (COCH<sub>3</sub>), 148.74 (COCH<sub>2</sub>CH<sub>2</sub>C), 133.79 (arom. CH), 132.16 (arom. CH), 128.66 (arom. CN), 125.87 (CCON), 95.06 (CCl<sub>3</sub>), 86.53 (NCHCHOH), 74.95 (CH<sub>2</sub>-TrOC), 60.67 (NCH), 58.24 (CH<sub>2</sub>CH<sub>2</sub>O), 56.04 (OCH<sub>3</sub>), 46.44 (NCH<sub>2</sub>), 35.24 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 28.59 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 23.08 (CH<sub>2</sub>CH<sub>2</sub>O)

Example 4(a):3-(7-methoxy-5-oxy(11aS)-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)-1-perhydro-1-pyrrolyl-1-propanone (161) (see Figure 22)

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3-(11-Hydroxy-7-methoxy-5-oxo-10-(2,2,2-trichloroethyloxocarbonylamino)-(11aS)-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[2,1-a][1,4]diazepin-8-yloxy-1-perhydro-1-pyrrolyl-1-propanone (160)

To a solution of 159 (100 mg, 0.196 mmol) in dichloromethane was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (44 mg, 0.23 mmol) and 4-(dimethylamino)pyridine (5 mg, 0.04

mmol) and the solution stirred for 1h. To the reaction was added pyrrolidine (16.36 mg, 0.23 mmol) and the reaction stirred for a further 2h. The solvent was then removed in vacuo and the compound purified by flash chromatography eluting-with 5% methanol in dichloromethane to give the product as a yellow oil, 5 yield = 56 mg, 51%.  $^{1}$ H NMR (CDCl<sub>3</sub>):  $\delta$  7.25 (OCCH), 6.90 (s, 1H, MeOCCHC), 5.66 (d, 1H, J = 5.49 Hz, TrOC-CH<sub>2</sub>), 5.16 (d, 1H, J =12.09 Hz, Troc-CH<sub>2</sub>), 4.84-4.74 (m, 2H, CHOH, C11aH), 4.35-4.23  $(m, 2H, CH_2CH_2O), 3.90 (s, 3H, OCH_3), ), 3.73-3.67 (m, 1H, NCH),$ 3.53-3.44 (m, 6H C-ring NCH<sub>2</sub>, pyrrolidine-  $N(CH_2)_2$ ), 2.92-2.76 10 (m, 2H CH<sub>2</sub>CH<sub>2</sub>O), 2.11-1.85 (8H, C-ring NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub> + pyrrolidine- $NCH_2CH_2CH_2$ );  $^{13}C-NMR$  (CDCl<sub>3</sub>):  $\delta$  168.62 (amide CO), 167.05 (CON), 154.31 (OCO), 149.94 (COCH<sub>3</sub>), 148.56 (COCH<sub>2</sub>CH<sub>2</sub>), 127.76 (arom. CN), 125.95 (CCON), 114.14 (arom. CH), 110.49 (arom. CH), 95.04  $(CCl_3)$ , 86.48 (NCHCHOH), 74.98  $(CH_2-TROC)$ , 65.15  $(CH_2CH_2O)$ , 60.20 15 (NCH), 56.13 (OCH<sub>3</sub>), 46.85, 46.44, 45.76, 34.47, 28.60, 26.02, 24.42 (various N-(X)CH<sub>2</sub>), 23.04 (CH<sub>2</sub>CH<sub>2</sub>O); FABMS m/z (relative intensity) 564 (M<sup>+</sup> 1), 550(3), 549 (2), 548 (8), 547 (2), 546 (8), 279 (2), 192 (4), 126 (18), 98 (6).

3-(7-methoxy-5-oxy(11aS)-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)-1-perhydro-1-pyrroly1-1-propanone (161)

Method A. To a solution of 160 (100 mg, 0.164 mmol) in dichloromethane (5 mL) was added 1-(3-dimethylaminopropyl)-3- ethylcarbodiimide hydrochloride (38 mg, 0.2 mmol) and pyrrolidine (14 mg, 0.2 mmol) and the reaction stirred for 18 h. The mixture was then dilute with dichloromethane (100 mL) and washed with

water (3 x 50 mL), saturated sodium bicarbonate solution (3 x 50 mL) and brine (50 mL). The solvent was removed *in vacuo* and the product purified by flash chromatography eluting with 5% methanol in dichloromethane to give the product 161 as a white solid (yield 26.3 mg, 40%)

Method B. To a solution of 160 (100 mg, 0.164 mmol) in dichloromethane (5 mL) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (38 mg, 0.2 mmol) and the reaction stirred for 3 hours. The reaction was then treated with tetrabutylammonium fluoride (200  $\mu$ L of a 1.0 M solution in THF, 0.2 mmol) and stirred for 30 min. The reaction was then treated with pyrrolidine (14 mg, 0.2 mmol) and stirred for 18 h. The mixture was then dilute with dichloromethane (100 mL) and washed with water (3 x 50 mL), saturated sodium bicarbonate solution (3 x 50 mL) and brine (50 mL). The solvent was removed in vacuo and the product purified by flash chromatography eluting with 5% methanol in dichloromethane to give the product 161 as a white solid (yield = 54.2 mg, 82%)

Method C: To a solution of 160 (56 mg, 0.1 mmol) in THF (3 mL) was added 1 M ammonium acetate solution (2 mL) and the reaction mixture stirred. To the solution was added 10% Cd/Pb couple (0.5 mmol, 62.4 mg) and the reaction was stirred for 90 min. The reaction was filtered and diluted with ethyl acetate (20 mL). The solution was dried with magnesium sulphate and the solvent removed in vacuo. the product as then purified by flash chromatography eluting with 5% methanol in dichloromethane to

give the compound as a white solid (yield = 21 mg, 56%). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.66 (m, 1H, J = 4.39 Hz, N=CH), 7.50 (s, 1H, arom. CH), 6.88 (s, 1H arom. CH), 4.42 (t, 2H, J = 6.96 Hz, OOCCH<sub>2</sub>CH<sub>2</sub>), 3.92 (s, 3H, OCH<sub>3</sub>), 3.90-3.44 (m, 5H, pyrrolidine CH<sub>2</sub>+NCH), 2.87 (t, 2H, 5.96 Hz, OOCCH<sub>2</sub>CH<sub>2</sub>), 2.28-2.33 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>), 2.10-1.87 (m, 8H, C-ring +pyrrolidine CH<sub>2</sub>). 168.58 (amide CO), 164.65 (CON), 162.43 (imine CH), 150.52 (COCH<sub>3</sub>), 147.61 (COCH<sub>2</sub>CH<sub>2</sub>), 140.76 (arom. CN), 120.33 (CCON), 111.54 (arom. CH), 110.61 (arom. CH), 65.20 (COCH<sub>2</sub>CH<sub>2</sub>), 56.21 (COCH<sub>3</sub>), 53.7 (NCH), 46.77, 46.67, 45.69, 34.40, 29.62, 26.06, 24.54, (CH<sub>2</sub>), 24.19 (COCH<sub>2</sub>CH<sub>2</sub>) MS (EI): m/e (relative intensity): 371 (M<sup>+</sup>, 10), 246 (10), 245 (5), 231 (3), 126 (18), 98 (2), 70 (5), 55 (3); HRMS calcd. for C<sub>20</sub>H<sub>15</sub>O<sub>4</sub>N<sub>3</sub> = 371.1845, found 371.1788.

Example 4(b): 3-(7-methoxy-5-oxy(11aS)-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)-1-piperidino-1-propanone (163) (see Figure 22)

3-(11-Hydroxy-7-methoxy-5-oxo-10-(2,2,2-

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trichloroethyloxocarbonylamino) - (11aS) -2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[2,1-a][1,4]diazepin-8-yloxy-1-perhydro-1-piperidino-1-propanone (162)

To a solution of 159 (100 mg, 0.196 mmol) in dichloromethane was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (44 mg, 0.23 mmol) and 4-(dimethylamino)pyridine (5 mg, 0.04

mmol) and the solution stirred for 1h. To the reaction was added piperidine (25  $\mu$ L, 0.23 mmol) and the reaction stirred for a The solvent was then removed in vacuo and the compound purified by flash chromatography eluting with 5% methanol in dichloromethane to give the product as a yellow oil, 5 yield = 94 mg, 84%).  $^{1}H-NMR$  (CDCl<sub>3</sub>):  $\delta$  7.25 (s,1H, OCCHCN), 6.90 (s, 1H, MeOCCHC), 5.65 (d, 1H, J = 9.71 Hz, TrOC-CH<sub>2</sub>), 5.17 (d, 1H, J = 11.94 Hz, TrOC-CH<sub>2</sub>), 4.37-4.24 (m, 4H, CHOH + CH<sub>2</sub>CH<sub>2</sub>O), 3.91 (s, 3H, OCH<sub>3</sub>), 3.73-3.67 (m, 1H, NCH), 3.54-3.45 (m, 6H,  $NCH_2$  piperidine- $N(CH_2)_2$ , 2.99-2.83 (m, 2H,  $CH_2CH_2O$ ), 2.13-2.00 10 (m, 4H,  $NCH_2CH_2CH_2$ ) 1.67-1.56 (m, 6H, piperidine- $CH_2$ ); <sup>13</sup>C NMR  $(CDCl_3): \delta 168.22 \text{ (amide CO)}, 167.11 (CON), 154.38 (OCO), 149.96$ (COCH<sub>3</sub>), 148.57 (COCH<sub>2</sub>CH<sub>2</sub>), 127.74 (arom. CN), 125.94 (CCON), 114.19 (arom. CH), 110.44 (arom. CH), 95.02 (CCl<sub>3</sub>), 86.38 (NCHCHOH), 74.96 (CH<sub>2</sub>-TROC), 65.38 (CH<sub>2</sub>CH<sub>2</sub>O), 60.33 (NCH), 56.0815  $(OCH_3)$ , 46.77 , 46.44, 42.75, 32.73, 28.60, 26.33, 25.48, 24.44 (various  $N-(X)CH_2$ ), 23.05 ( $CH_2CH_2O$ ); MS (EI) m/z (relative intensity): = 579 (1), 577 (1), 331 (1), 278 (1), 246 (1), 192 (4), 140 (32), 113 (2), 112 (2), 97 (1), 84 (3), 77 (3), 70 (7), 69 (4), 55 (4), HRMS calcd. for  $C_{24}H_{30}N_3O_7Cl_3 = 579.1120$  found 20 579.1066

3-(7-methoxy-5-oxy(11aS)-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)-1-piperidino-1-propanone (163)

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To a solution of 162 (94 mg, 0.162 mmol) in THF (3 mL) was added 1 M ammonium acetate solution (2 mL) and the reaction mixture stirred. To the solution was added 10% Cd/Pb couple (0.81 mmol,

100 mg) and the reaction was stirred for 90 min. The reaction was filtered and diluted with ethyl acetate (20 mL). solution was dried with magnesium sulphate and the solvent removed in vacuo. the product as then purified by flash chromatography eluting with 5% methanol in dichloromethane to 5 give the compound as a white solid (yield = 25 mg, 39%). H NMR  $(CDCl_3)$ :  $\delta$  7.67 (d, 1H, J = 4.4 Hz, N=CH), 7.51 (s, 1H, OCCHCN), 6.89 (s, 1H, MeOCCHC), 4.42 (t, 2H, J = 7.14 Hz,  $CH_2CH_2O$ ), 3.93  $(s, 3H, OCH_3), 3.90-3.44$  (m, 5H, NCH, NCH<sub>2</sub>, piperidine-N(CH<sub>2</sub>)<sub>2</sub>), 2.73 (t, 2H, J = 7.32 Hz  $CH_2CH_2O$ ), 2.33-2.29 (m, 2H, C-ring 10  $CH_2$ ), 2.11-2.02 (m, 2H, C-ring  $CH_2$ ), 1.62-1.59 (m, 6H, piperidine  $CH_2$ ),  $^{13}C$  NMR (CDCl<sub>3</sub>):  $\delta$  168.19 (amide CO), 164.66 (imine CH), 162.43 (CON), 150.52 (COCH<sub>3</sub>), 147.61 (COCH<sub>2</sub>CH<sub>2</sub>), 140.70 (arom. CN), 120.31 (CCON), 111.51 (arom. CH), 110.58 (arom. CH), 65.44  $(CH_2CH_2O)$ , 56.11  $(OCH_3)$ , 53.73 (NCH), 46.70, 46.39, 42.69, 32.72, 15 29.62, 26.38, 25.52, 24.40 (various  $N-(X)CH_2$ ), 24.19 ( $CH_2CH_2O$ ); MS (EI): m/e (relative intensity): 385 ( $M^+$ , 6), 246 (8), 245 (3), 231 (3), 140 (15), 138 (5), 97 (5), 84 (3); HRMS calcd. for  $C_{21}H_{27}O_4N_3 = 385.2002$ , found 385.2058.

Example 4(c): 1, (2,3-dihydro-1H-indoly1)-3-(7-methoxy-5
oxy(11aS)-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2
a][1,4]diazepin-8-yloxy)-1-propanone (165) (see Figure 22)

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1-(2,3-Dihydro-1H-1-indolyl)-3-(11-hydroxy-7-methoxy-5-oxo-10-(2,2,2-trichloroethyloxocarbonylamino)-(11aS)-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[2,1-a][1,4]diazepin-8-yloxy-1-propanone (164)

To a solution of 159 (100 mg, 0.196 mmol) in DMF was added 1-(3dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (44 mg, 0.23 mmol) and 4-(dimethylamino)pyridine (5 mg, 0.04 mmol) and 10 the solution stirred for 1h. To the reaction was added indoline (27.4 mg, 0.23 mmol) and the reaction stirred for a further 8h. The solvent was then removed in vacuo and the compound purified by flash chromatography eluting with 5% methanol in dichloromethane to give the product as a yellow oil (yield = 71 15 mg, 61%).  $^{1}H-NMR$  (CDCl<sub>3</sub>):  $\delta$  1.99-2.12 (m, 4H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 3.20  $(t, J = 8.42 \text{ Hz}, CH_2CH_2O), 3.71-5.00, (m, 4H, NCH_2, NCH, CHOH),$ 3.89 (s, 3H, OC $\mathbf{H_3}$ ), 4.18-4.09 (m, 2H, indole-CH $_2$ ), 4.27 (d, 2H, J = 11.90 Hz, indole-CH<sub>2</sub>), 4.43 (t, J = 6.23 Hz,  $CH_2CH_2O$ ), 5.16 (d, 1H, J = 11.91 Hz, TrOC-CH<sub>2</sub>), 5.30 (s, 1H, OH), 5.66 (d, 1H, J =20 9.89 Hz,  $TrOC-CH_2$ ), 7.20-6.93 (m, 5H, indole-CH, arom CH), 8.18 (d, 1H, J = 8.25 Hz, indole-CH);  $^{13}\text{C-NMR}$  (CDCl<sub>3</sub>):  $\delta$  168.24 (CON), 166.97 (CON), 154.36 (OCO), 149.91, COCH<sub>3</sub>), 148.65 (COCH<sub>2</sub>CH<sub>2</sub>),

132.14, 131.99 (indolyl ring junction), 128.61, 128.43 (indole-CH), 127.52, (arom. CN), 124.61 (CCON), 114.20 (arom. CH), 110.58 (arom. CH) 95.02 (CCl<sub>3</sub>), 86.43 (NCHCHOH), 75.01 ((TrOC-CH), 64.89 (CH<sub>2</sub>CH<sub>2</sub>O), 60.13 (NCH), 56.11 (OCH<sub>3</sub>), 48.11 (indole-CH<sub>2</sub>), 46.43 (NCH<sub>2</sub>), 35.64, 28.64, 27.97, (CH<sub>2</sub>), 23.03 (CH<sub>2</sub>CH<sub>2</sub>O); MS (EI) m/z (relative intensity): = 595 (M<sup>+</sup> 1), 415 (1), 365 (1), 246 (2), 192 (13), 174 (11), 173 (7), 119 (17), 118 (10), 70 (13).

Iso-indoline (2,3,-dihydro-1H-isoindole) <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.22 10 (m, 4H, arom CH), 4.26 (s, 4H, CH<sub>2</sub>), 4.08 (bs, 1H, NH), <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 140.37, 140.36 (ring junctions), 127.15, 126.90, 122.60, 122.51, 122.33 (arom. CH), 52.31 (CH<sub>2</sub>).

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1,(2,3-dihydro-1H-indoly1)-3-(7-methoxy-5-oxy(11aS)-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)-1-propanone (165)

To a solution of 164 (71 mg, 0.116 mmol) in THF (3 mL) was added 1 M ammonium acetate solution (2 mL) and the reaction mixture stirred. To the solution was added 10% Cd/Pb couple (0.58 mmol, 72 mg) and the reaction was stirred for 90 min. The reaction was filtered and diluted with ethyl acetate (20 mL). The solution was dried with magnesium sulphate and the solvent removed in vacuo. The product as then purified by flash chromatography eluting with 5% methanol in dichloromethane to give the compound as a white solid (yield = 26 mg, 54%).  $^{1}$ H NMR (CDCl<sub>3</sub>):  $\delta$  7.66 (d, 1H, J = 4.58 Hz, CH=N), 7.50 (s, arom. CH), 7.19 (m, 4H indolyl arom. CH), 6.91 (s, 1H, arom. CH), 4.48 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>O), 4.18-4.19 (m, 2H, indolyl CH<sub>2</sub>), 3.91 (s, 3H, OCH<sub>3</sub>), 3.88-3.44 (m,

3H, NCH, +indolyl CH<sub>2</sub>), 3.02 (t, 2H, J = 6.6 Hz, CH<sub>2</sub>CH<sub>2</sub>O), 2.30-2.28 (m, 2H, NCH<sub>2</sub>), 2.17-2.05 (m, 4H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 168.31 (amide CO), 164.61 (CON), 162.47, (imine CH), 147.59 (COCH<sub>2</sub>CH<sub>2</sub>), 140.70 (arom. CN), 127.53, 124.59, 123.87, (indolyl arom. CH), 120.44 (CCON), 117.03 (indolyl arom. CH), 11.56 (arom. CH), 110.61 (arom. CH), 64.80 (COCH<sub>2</sub>CH<sub>2</sub>), 56.14 (COCH<sub>3</sub>), 53.70 (NCH), 48.11, 46.69, 35.50, 29.60, 28.67, 28.00 (CH<sub>2</sub>), 24.19 (COCH<sub>2</sub>CH<sub>2</sub>).

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Example 4(d): 1,(2,3-dihydro-1H-2-isoindolyl)-3-(7-methoxy-5
oxy(11aS)-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2
a][1,4]diazepin-8-yloxy)-1-propanone (167) (see Figure 22)

1-(2,3-dihydro-1H-2-isoindoly1)-3-(11-hydroxy-7-methoxy-5-oxo-10-(2,2,2-trichloroethyloxocarbonylamino)-(11aS)-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[2,1-a][1,4]diazepin-8-yloxy-1-propanone (166)

To a solution of **159** (100 mg, 0.196 mmol) in DMF was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (44 mg, 0.23 mmol) and 4-(dimethylamino)pyridine (5 mg, 0.04 mmol) and the solution stirred for 1h. To the reaction was added indoline (27.4 mg, 0.23 mmol) and the reaction stirred for a further 8h. The solvent was then removed *in vacuo* and the compound purified

by flash chromatography eluting with 5% methanol in dichloromethane to give the product as a yellow oil (yield = 75 mg, 64%).  $^{1}\text{H-NMR}$  (CDCl<sub>3</sub>):  $\delta$  7.29-7.20 (m, 5H, isoindole arom. + arom.CH), 6.91 (s, 1H, arom CH), 5.66 (d, 1H, J = 9.7 Hz, TrOC- $CH_2$ ) 5.30 (s, 1H, OH), 5.19 (d, 1H, J = 9.7 Hz,  $TrOC-CH_2$ ), 4.94 5 (m, 2H, isoindolyl CH<sub>2</sub>), 4.79 (s, 2H, isoindolyl CH<sub>2</sub>), 4.38 (t,2H, J = 6.42 Hz,  $CH_2CH_2O)$ , 4.25, (d, 1H, J = 11.91 Hz, C11-H), 3.81-3.40 (2H, NCH<sub>2</sub>), 3.03-2.85 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>O), 2.11-1.98 (m, 4H,  $NCH_2CH_2CH_2$ );  $^{13}C-NMR$  (CDCl<sub>3</sub>):  $\delta$  169.17 (CON), 167.02 (CON), 154.27 (OCO), 149.91 (COCH<sub>3</sub>), 148.64 (COCH<sub>2</sub>CH<sub>2</sub>), 136.19, 136.1110 (isoindolyl ring junction), 128.61, 127.88 (isoindolyl CH), 127.78 (arom. CN), 127.58, (CCON), 114.28 (arom. CH), 110.54 (arom. CH), 95.09 (CCl<sub>3</sub>), 86.51 (NCHCHOH), 74.98 (TrOC-CH<sub>2</sub>), 65.21 ( $CH_2CH_2O$ ), 60.23 (NCH), 56.05 (OCH<sub>3</sub>), 52.14, 52.81 (isoindolyl  $CH_2$ ), 46.43, (NCH<sub>2</sub>), 34.31, 29.68, 28.60 (NxCH<sub>2</sub>), 15 23.03 ( $CH_2CH_2O$ ); FABMS m/z (relative intensity): = 612 (1), 596 (1), 594 (1), 279 (1), 192 (1), 174 (8), 146 (5), 118 (13), 91 (2), 55 (3). FABHRMS found compound minus OH i.e.  $C_{27}H_{27}N_3O_6Cl_3 =$ 595.1044

1,(2,3-dihydro-1H-2-isoindoly1)-3-(7-methoxy-5-oxy(11aS)2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8yloxy)-1-propanone (167)

To a solution of 166 (75 mg, 0.122 mmol) in THF (3 mL) was added 1 M ammonium acetate solution (2 mL) and the reaction mixture stirred. To the solution was added 10% Cd/Pb couple (0.61 mmol, 76 mg) and the reaction was stirred for 90 min. The reaction was filtered and diluted with ethyl acetate (20 mL). The solution

was dried with magnesium sulphate and the solvent removed in vacuo. The product was then purified by flash chromatography eluting with 5% methanol in dichloromethane to give the compound as a white solid (yield = 42.6 mg, 83%).  $^{1}$ H NMR (CDCl<sub>3</sub>):  $\delta$  7.66 (d, 2H, J = 4.39 Hz, N=CH), 7.48 (s, 1H, arom. CH), 7.30 (s, 4H, 5 indolyl arom. CH), 6.89 (s, 1H, arom. CH), 4.48 (t, 3H, J = 6.59Hz, COCH<sub>2</sub>CH<sub>2</sub>), 3.84 (s, 3H, OCH<sub>3</sub>), 3.81-3.69 (m, 2H, indolyl $CH_2$ ), 3.61-3.51 (m, 1H, NCH), 2.97 (p, 5H, J = 6.9 Hz,  $CH_2CH_2O$ ), 2.32-2.28 (m, 2H, NCH<sub>2</sub>), 2.30-2.01 (m, 4H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>);  $^{13}$ C NMR  $(CDCl_3): \delta 169.29$  (amide CO), 164.66 (imine CH), 162.52 (CON), 10 150.45 (COCH<sub>3</sub>), 147.63 (COCH<sub>2</sub>CH<sub>2</sub>), 140.57, (arom. CN), 127.86, 127.56, 123.04, 122.62 (indolyl arom. CH), 120.38 (CCON), 111.52 (arom. CH), 110.53 (arom. CH), 65.16 (COCH<sub>2</sub>CH<sub>2</sub>), 56.06 (COCH<sub>3</sub>), 53.73 (NCH), 52.16, 50.64, 46.70, 34.22, 29.57 (CH<sub>2</sub>), 24.18 (COCH<sub>2</sub>CH<sub>2</sub>); MS (EI): m/e (relative intensity): 419 (M<sup>+</sup>, 21), 41615 (2), 415 (2), 246 (10), 245 (3), 231 (3), 174 (4); HRMS calcd. for  $C_{24}H_{25}O_4N_3 = 419.1845$ , found 419.1821.

### Examples 5 to 8 : Cytotoxicity Data

#### NCI In Vitro Cytotoxicity Studies

The National Cancer Institute (NCI), Bethesda, Maryland, USA has available an in vitro cytotoxicity screen which consists of approximately 60 human tumour cell lines against which compounds are tested at a minimum of five concentrations each differing 10-fold. A 48 hour continuous exposure protocol is used, where cell viability or growth is estimated with an SRB protein assay.

Method

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The test compounds were evaluated against approximately 60 human tumour cell lines. The NCI screening procedures were described in detail by Monks and co-workers (Monks, A et al., Journal of the National Cancer Institute, 1991, 83, 757). Briefly, cell suspensions were diluted according to the particular cell type and the expected target cell density (5000-40,000 cells per well based on cell growth characteristics), and added by pipette (100  $\mu L)$  into 96-well microtitre plates. The cells were allowed a preincubation period of 24 h at 37°C for stabilisation. Dilutions at twice the intended test concentration were added at time zero in 100  $\mu L$  aliquots to the wells. The test compounds were evaluated at five 10-fold dilutions ( $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$ and  $10^{-8}~\mu\text{M})\,.$  The test compounds were incubated for 48 h in 5%  ${\rm CO_2}$  atmosphere and 100% humidity. The cells were then assayed using the sulphorhodamine B assay. A plate reader was used to read the optical densities and a microcomputer processed the readings into  $LC_{50}$  values, which is the dosage required to kill half of the cells.

The results presented in examples 5 to 8 are  $LC_{50}$  values which are below 10 $\mu$ M, which is taken to be the dividing line between cytotoxicity and non-cytotoxicity.

NCI Hollow Fibre Assay for Preliminary In Vivo Testing

The Biological testing Branch of the Developmental Therapeutics Program of the NCI has adopted a preliminary in vivo screening

tool for assessing the potential anticancer activity of compounds identified by the large scale in vitro cell screen. For these assays, human tumour cells are cultivated in polyvinylidene (PVDF) hollow fibres, and a sample of each cell line is implanted into each of two physiologic compartments (intraperitoneal and subcutaneaous) in mice. Each test mouse received a total of 6 fibres (3 intraperitoneally and 3 subcutaneously) representing 3 distinct cancer cell lines. These mice are treated with potential antitumour compounds at each of 2 test doses by the intraperitoneal route using a QD x 4 treatment schedule. Vehicle controls consist of 6 mice receiving the compound diluent only. The fibre cultures are collected on the day following the last day of treatment. To assess anticancer effects, the viable cell mass is determined for each of the cell lines using a formazyn dye (MTT) conversion assay. From this, the %T/C can be calculated using the average optical density of compound treated samples divided by the average optical; density of the vehicle controls. In addition, the net increase in cell mass can be determined for each sample as a sample of fibre cultures are assessed for viable cell mass on the day of implantation into mice. Thus, the cytostatic and cytocidal capacities of the test compound can be assessed.

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Generally, each compound is tested against a minimum of 12 human cancer cell lines. This represents a total of 4 experiments

25 since each experiment contains 3 cell lines. The data are reported as %T/C for each of the 2 compound doses against each of the cell lines with separate values calculated for the

intraperitoneal and subcutaneous samples.

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Compounds are selected for further in vivo testing in standard subcutaneous xenograft models on the basis of several hollow fibre assay criteria. These include: (1) a %T/C of 50 or less in 10 of the 48 possible test combinations (12 cell lines X 2 sites X 2 compound doses); (2) activity at a distance (intraperitoneal drug/subcutaneous culture) in a minimum of 4 of the 24 possible combinations; and/or (3) a net cell kill of 1 or more of the cell lines in either implant site. To simplify evaluation, a points system has been adopted which allows rapid viewing of the activity of a given compound. For this, a value of 2 is assigned for each compound dose which results in a 50% or greater reduction in viable cell mass. The intraperitoneal and subcutaneous samples are scored separately so that criteria (1) and (2) can be evaluated. Compounds with a combined IP + SC score of 20, a SC score of 8 or a net cell kill of one or more cell lines are refereed for xenograft testing. This comparison indicated that there was a very low probability of missing an active compound if the hollow fibre assay were used as the initial in vivo screening tool. In addition to these criteria, other factors (e.g. unique structure, mechanism of action) may result in referral of a compound for xenograft testing without the compound meeting these criteria.

# Example 5: In Vitro Cytotoxicity of compounds of formula I

25 All of the compounds synthesised in example 1, were subjected to the NCI In Vitro Cytotoxicity study. The results (LC50; $\mu$ M) are

set out below, and are illustrated in Figure 23.

TUMOUR TYPE	CELL-LINE DESIGNATION	UP2003 ( <b>24</b> )	UP2051 ( <b>31</b> )	UP2052 ( <b>33</b> )	UP2065 ( <b>42</b> )
Lung	NCI-H23		9.3		
	NCI-H460	7.6		3.0	
	NCI-H522			3.1	
Colon	COLO 205	1.4			4.0
	HCC-2998	5.2	5.2	0.8	
	HCT-116			1.1	
	KM12	9.5			
CNS	SNB-75	6.0			
Melanoma	MALME-3M	0.7	5.1		4.7
	M14			2.7	
	SK-MEL-2		7.6	0.5	3.5
	UACC-62	0.7			
Renal	786-0			3.0	
	RXF 393		0.8		0.8
Breast	MDA-MB-435			l 	0.8

of the compounds tested, the above showed cytotoxicity against human lung, colon, CNS, melanoma, renal and breast cancer cell lines. Replacing the C-8 benzyloxy group in UP2003 (24) with a methoxy substituent (UP2065, 42) significantly changed the cytoxicity profile, activity was lost against lung, CNS, and colon cancer cell lines (only reduced activity against Colo 205 remained). However, additional cytotoxic activity was gained against the melanoma cell line SKMEL-2, the renal cell line RXF-393 and the breast cell line MDA-MB-435. Reduction of the ester moiety in UP2003 (24) to afford the alcohol UP2052 (33) resulted in increased activity in the lung cancer cell line NCI-460 and the colon cell line HCC-2998. Additional activity was registered against the lung cell line NCI-H522, the colon cell line HCT-116, the melanoma cell line M14 and the renal cancer cell line 786-0.

Interestingly, the acetylated analogue UP2051 (31) exhibited attenuated or abolished activity in these cell lines (e.g.  $7.6\mu M$  verses 0.5  $\mu M$  for UP2052 in the melanoma SK-MEL-2 cell line.

# Example 6(a): In Vitro Cytotoxicity of compounds of formula II

All of the compounds synthesised in example 2, were subjected to the NCI *In Vitro* Cytotoxicity study. The results (LC50; $\mu$ M) are set out below, and are illustrated in Figure 24.

TUMOUR TYPE	CELL-LINE DESIGNATION	UP2064 ( <b>74</b> )	UP2001 ( <b>80</b> )	UP2004 ( <b>70</b> )	UP2023 ( <b>64</b> )	UP2067 ( <b>172</b> )
Lung	NCI-H23			7.6		
	NCI-H226				9.1	
	NCI-H460		2.7			
	NCI-H522				5.2	5.0
Colon	COLO 205	0.6		3.9	5.8	5.8
	HCC-2998		0.099	5.5	7.0	
	KM12				7.1	
CNS	SF-539				9.4	6.8
	SNB-75		7.5			5.4
Melanoma	MALME-3M	0.9	0.073		7.8	7.4
	M14					0.8
	SK-MEL-2	1.7			7.4	, 
	SK-MEL-28	2.6			8.4	6.6
	SK-MEL-5			7.8	6.0	
	UACC-257	7.4		7.3		
	UACC-62	0.6	0.077	5.3	7.2	3.0
Renal	RXF 393	0.8			6.1	0.8
Breast	MDA-MB-435	2.3			7.6	0.8
	MDA-N			9.0	6.6	0.6

Of the compounds tested, the above listed exert their cytotoxic effect ( $LC_{50}$ ) most strongly in the Lung, Colon, CNS. Melanoma, Renal and Breast cell line panels. Within the group, it is apparent that exchanging a C-8 benzyloxy substituent (UP2004, 70) for a methoxy group (UP2064, 74) results in increased activity in

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the Melanoma panel. The methoxy analogue is more potent and acts against a greater number of cell lines. The methoxy analogue also exhibits improved activity against the colon cancer cell line Colo 205 and, in addition, the methoxy analogue exhibits activity against the renal cell line RXF-393 which is not observed with the benzyloxy compound. Replacing the electron rich dimethoxy A-ring with for an iodo substituted aromatic ring (UP2023, 64) resulted in slight attenuation of activity in some cell lines, but the analogue showed activity against a wider spread of cell lines (i.e. 5 melanoma cell lines against only 3 for the benzyloxy analogue). Changing the nature of the C-ring ex0-unsaturation from an alkene to a ketone (UP2067, 172) lead to additional activity against the breast cancer cell line MDA-MB-435, renal cell line RXF-393, the melanomas MALME-3M, M14, SKMEL-28, the CNS cancers SF-539 and SNB-75 and against the lung cell line NCI-H522.

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The PBD dimer UP2001 (80) exhibited potent and selective cytoxicity activity against the lung cancer cell line NCI-H460, the colon cell line HCC-2998, the CNS cancer cell line SNB-75 and the melanoma cell lines MALME-3M (very potent, 0.08  $\mu$ M) and UACC-62 (very potent, 0.07  $\mu$ M), which may be attributable to its ability to cross link DNA.

## Example 6(b): Hollow Fibre Assay on Compounds of formula II

Two of the compounds tested underwent the NCI Hollow Fibre Assay, and the results are presented below.

	UP2001 ( <b>80</b> )	UP2004 ( <b>70</b> )
IP score	40	8
SC score	14	10
Total score	54	18
Cell Kill	Y	N

5 UP2001 (80) and UP2004 (70) were subjected to the NCI Hollow
Fibre assay described above. UP2001 has been selected for
xenograft studies based on its combined IP + SC score (54) which
was greatly in excess of 20, and its SC score which was higher
than 8. UP2004 has been selected on the basis of its SC score,
10 it being higher than 8.

# Example 7: In Vitro Cytotoxicity of compounds of formula III

All of the compounds synthesised in example 3, were subjected to the NCI In Vitro Cytotoxicity study. The results (LC50; $\mu$ M) are set out below, and are illustrated in Figure 25.

TUMOUR TYPE	CELL-LINE DESIGNATION	UP2026 ( <b>136</b> )	UP2027 (138)	UP2028 ( <b>151</b> )	UP2068 ( <b>96</b> )
Lung	NCI-H522	7.8	8.0	0.8	8.5
Colon	COLO 205	8.8		5.0	
	HCC-2998	6.4			
	KM12			8.8	
CNS	SNB-75			8.2	
Melanoma	MALME-3M	6.1		5.7	8.3
	LOX IMVI				9.7
	M14	7.8			6.5
	SK-MEL-2	7.4	9.5	5.4	8.1
	SK-MEL-28	7.1		8.1	9.6
	SK-MEL-5	9.0			

7.7

UACC-257

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	UACC-62	6.6			
Renal	RXF 393	7.6	6.6	0.7	6.3
Breast	HS 578T			9.2	
	MDA-MB-435	6.3		7.2	8.3
	MDA-N				6.3

The C-7-phenyl substituted compound UP2026 (136) showed cytotoxicity against cell lines in the human lung, colon, melanoma, renal and breast cancer cell line panels. Interestingly, unlike other PBDs the molecule was inactive in the CNS cell line panel. However, UP2026 (136) was active against nearly all the members of the melanoma panel. Inclusion of a methoxy group in the C7 aryl moiety (138) resulted in increased selectivity as cytoxicity was only observed in the lung cell line NCI-H522, the melanoma cell line SKMEL-2 and the renal cell line RXF-393. Introduction of a nitro group at C7 completely abolished cytotoxic activity, however, it seems likely that activity would be restored once the nitro group is reduced to an amine; in this way UP2029 (140) might prove to be a useful prodrug. The C8 amino substituted PBD (UP2028, 151) showed good activity in the lung, colon, CNS, melanoma, renal and breast cell line panels. On the other hand the trimethoxy PBD (UP2068, 96) was only active in the lung, melanoma, renal and breast cell line panels.

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## Example 8: In Vitro cytotoxicity of compounds of formula IV

The compounds synthesised in example 4, were subjected to the NCI In Vitro Cytotoxicity study. The results (LC50; $\mu$ M) are set out below, and are illustrated in Figure 26.

TUMOUR	CELL-LINE	UP2005	UP2008
TYPE	DESIGNATION	(161)	(167)
Lung	NCI-H23		8.9
	NCI-H522	8.7	
Colon	HCC-2998		8.1
CNS	SF-295	8.8	
	SF-539	7.7	
Melanoma	MALME-3M	7.5	6.8
	LOX IMVI	9.2	
	M14	6.2	8.4
,-	SK-MEL-2	7.6	6.5
	SK-MEL-28	6.5	
	UACC-257		7.1
Renal	RXF 393	6.8	

Two of the four C8 PBD amides, UP2005 (161) and UP2008 (167), demonstrated cytotoxicity (LC<sub>50</sub>) in the NCI assay. UP2005 (161)

10 showed selectivity for the lung, CNS, melanoma and renal cancer cell in panels. The compound was particularly active in the melanoma panel exhibiting cytotoxicity against 5 out of the 8 melanoma cell lines. UP2008 (167) revealed a slightly different profile being active in the lung, colon, and melanoma panels.

15 Again the molecule was particularly active in the melanoma panel.

# Example 9: Further results for PBD dimer SJG-136 (UP2001, 80)

The compound synthesized in example 2(d) (SJG-136, 80) underwent some further assays.

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The first assay, which is described in G.B.Jones, et al., Anti-Cancer Drug Des., 1990, 5, 249, which is incorporated herein by reference, determines the effect of the test compound on the helix melting temperature of DNA. This assay is designed to give an indication of the strength and extent of cross-linking of the DNA strands by the test compound (i.e. a measure of the stabalisation of the DNA upon ligand binding).

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The melting temperature is determined for a 1:5 molar ratio of [ligand][DNA], where the calf thymus DNA concentration is 100 mM in aqueous sodium phosphate buffer (10 mM sodium phosphate + 1 mM EDTA, pH  $7.00 \pm 0.01$ ). For calf thymus DNA at pH  $7.00 \pm 0.01$ , the melting temperature is  $67.83 \pm 0.06$  °C (mean value from 30 separate determinations).

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For a 1:5 molar ratio of [PBD]:[DNA], the PBD dimer 80 elevates the helix melting temperature ( $\Delta T_m$ ) of calf thymus DNA by an unprecedented 33.6 °C after incubation for 18 hours at 37 °C. Under identical conditions, the C-ring-unsubstituted dimer DSB-120:

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#### **DSB-120**

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provides a  $\Delta T_m$  of 15.1°C, demonstrating the extraordinary effect of introducing C2/C2'-unsaturation. In common with other PBD dimers, 80 exerts most of its effect upon the GC-rich or high temperature regions of the DNA melting curves. In a similar fashion to DSB-120, it provides some 60-80% of its stabilising effect without prior incubation, suggesting a kinetic effect in the PBD reactivity

profile. However, the comparative  $\Delta T_m$  curves show that, on a concentration basis alone, SJG-136 is  $\geq$  10-fold more effective than DSB-120. Even at a [PBD]:[DNA] molar ratio of 1:100, SJG-136 still exhibits significantly better DNA binding affinity than the monomer tomaymycin at a 1:5 [PBD][DNA] molar ratio.

**Tomamycin** 

The results for a [PBD]:[DNA] ratio of 1:5 are summarised in the table below (All  $\Delta Tm$  values  $\pm$  0.1-0.2 °C)

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Compound	Induced $\Delta T_{\rm m}$ (°C) after incubation at 37 °C for						
	0 h	4 h	18 h				
SJG-136 (80)	25.7	31.9	33.6				
DSB-120	10.2	13.1	15.1				
Tomamycin	0.97	2.38	2.56				

The data presented in the above table show that SJG-136 (80) is the most potent DNA-stabilising agent known to date according to this particular assay.

The second assay determined the cytotoxicity of SJG-136 (80) in the human ovarian carcinoma cell line A2780 and its cisplatin-resistant subline A2780cisR, and compared this data with the cytotoxicity of the related dimer DSB-120 (see above) and Cisplatin. Relative to the parental line, the A2780cisR subline is known to have elevated GSH levels, an increased level of repair of DNA-cisplatin adducts, and a decreased ability to uptake cisplatin (M.Smellie, et al., Br. J. Cancer, 1994, 70, 48).

The results, which were obtained by incubating the cells with the compounds for 96 hours at 37°C, and assessing the cell number using Sulforhodamine B, are presented in the table below:

	$1C_{50}^{a}~(\mu M)$ for A2780 A2780cis <sup>R</sup> RF <sup>b</sup>					
SJG-136 (80)	0.0000225	0.000024	1.1			
DSB-120	0.0072	0.21	29.2			
Cisplatin	0.265	8.4	32			

a Dose of compounds required to inhibit cell growth by 50% compared with control

b RF is the resistance factor (IC50 resistant/parent)

The  $IC_{50}$  value for **80** in the A2780 cell line is only 23 pM, representing a 320-fold increase in cytotoxicity compared to DSB-120 ( $IC_{50} = 7.2 \text{ nM}$ ). More interestingly, whereas DSB-120 has a reduced potency in the cisplatin-resistant A2780cisR ( $IC_{50} = 0.21 \text{ mM}$ ), SJG-136 is almost 9,000-fold more potent in this cell line with a similar  $IC_{50}$  value (24 pM) to that in the normal A2780, giving a Resistance Factor of 1.1. The fact that both DSB-120 and cisplatin give Resistance Factors of 29.2 and 32, respectively, across this pair of cell lines suggests that SJG-136 may have potential in the treatment of cisplatin-refractory disease.

#### CLAIMS

A compound of the formula Ia or Ib:

wherein:

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A is  $CH_2$ , or a single bond; 5  $R_2$  is selected from: R, OH, OR,  $CO_2H$ ,  $CO_2R$ , COH, COR,  $SO_2R$ , CN;  $R_6$ ,  $R_7$  and  $R_9$  are independently selected from H, R, OH, OR, halo, amino, NHR, nitro, Me<sub>3</sub>Sn; where R is a lower alkyl group having 1 to 10 carbon atoms, or an aralkyl group of up to 12 carbon atoms, whereof the alkyl group 10 optionally contains one or more carbon-carbon double or triple bonds, which may form part of a conjugated system, or an aryl group of up to 12 carbon atoms; and is optionally substituted by one or more halo, hydroxy, amino, or nitro groups, and optionally containing one or more hetero atoms which may form part of, or be, a 15 functional group; and  $R_{8}$  is selected from H, R, OH, OR, halo, amino, NHR, nitro,  ${\rm Me_3Sn}$ , where R is as defined above, or the compound is a dimer with each monomer being the same or different and being of formula Ia or Ib, where the  $R_{\theta}$  groups of the monomers form together a bridge 20 having the formula -X-R'-X- linking the monomers, where R' is an alkylene chain containing from 3 to 12 carbon atoms, which chain may be interrupted by one or more hetero-atoms and/or aromatic rings and may contain one or more carbon-carbon double or triple bonds, and

each X is independently selected from O, S, or N; except that in a compound of formula Ia when A is a single bond, then  $R_2$  is not CH=CH(CONH<sub>2</sub>) or CH=CH(CONMe<sub>2</sub>).

- 2. A compound of formula Ia according to claim 1, with the proviso that when A is a single bond, then  $R_2$  is not  $CH=CR^AR^B$ , where  $R^A$  and  $R^B$  are independently selected from H,  $R^C$ ,  $COR^C$ ,  $CONH_2$ ,  $CONHR^C$ ,  $CONR^C_2$ , cyano or phosphonate, where  $R^C$  is an unsubstituted alkyl group having 1 to 4 carbon atoms.
- 3. A compound according to either claim 1 or claim 2, wherein A is  $CH_2$ .
- 10 4. A compound according to claim 3, wherein  $R_2$  is  $CO_2H$ ,  $CO_2R$ ,  $CH_2OH$ , or  $CH_2OR$ .
  - 5. A compound according to claim 4, wherein  $\rm R_2$  is  $\rm CO_2^{t}Bu$ ,  $\rm CH_2OH$ , or  $\rm CH_2OAc$ .
- 6. A compound according to any one of the preceding claims wherein  $R_6$ ,  $R_7$  and  $R_9$  and, unless the compound is a dimer,  $R_8$  are independently selected from H and OR.
- 7. A compound according to claim 6, wherein  $R_6$ ,  $R_7$  and  $R_9$  and, unless the compound is a dimer,  $R_8$  are independently selected from H, OMe and OCH<sub>2</sub>Ph.
- 8. A compound according to claim 6, wherein  $R_7$  and, unless the compound is a dimer,  $R_8$  are OR, and  $R_6$  and  $R_9$  are H.
  - 9. A compound according to claim 8, wherein  $R_7$  and, unless the compound is a dimer,  $R_8$  are independently either OMe or OCH<sub>2</sub>Ph.
- 30 10. A compound according to any one of the preceding claims of formula Ia.
  - 11. A compound according to any one of the preceding claims which is a dimer, wherein the dimer bridge is of the formula  $-0-(CH_2)_p-0-$ , where p is from 1 to 12.
    - 12. A compound of formula II:

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wherein:

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 ${\rm R'}_2$  is selected from: O, CHR" $_2$ , where  ${\rm R''}_2$  is selected from H, R,  ${\rm CO}_2{\rm R}$ , COR, CHO,  ${\rm CO}_2{\rm H}$ , halo;

 $R_6$ ,  $R_7$  and  $R_9$  are independently selected from H, R, OH, OR, halo, amino, NHR, nitro, Me<sub>3</sub>Sn;

where R is a lower alkyl group having 1 to 10 carbon atoms, or an aralkyl group of up to 12 carbon atoms, whereof the alkyl group optionally contains one or more carbon-carbon double or triple bonds, which may form part of a conjugated system, or an aryl group of up to 12 carbon atoms; and is optionally substituted by one or more halo, hydroxy, amino, or nitro groups, and optionally containing one or more hetero atoms which may from part of, or be, a functional group;

and  $R_8$  is selected from H, R, OH, OR, halo, amino, NHR, nitro,  $Me_3Sn$ , where R is as defined above or the compound is a dimer with each monomer being the same or different and being of formula II, where the  $R_8$  groups of the monomers form together a bridge having the formula -X-R'-X- linking the monomers, where R' is an alkylene chain containing from 3 to 12 carbon atoms, which chain may be interrupted by one or more hetero-atoms and/or aromatic rings and may contain one or more carbon-carbon double or triple bonds, and each X is independently selected from O, S, or N; except that:

- (i) when R'  $_2$  is CH-Et, and R  $_6,\ R_8$  and R  $_9$  are H, R  $_7$  is not sibirosamine pyranoside; and
- (ii) when  ${\rm R'}_2$  is CH-Me, and  ${\rm R}_6$  and  ${\rm R}_9$  are H,  ${\rm R}_7$  and  ${\rm R}_8$  are not both H or both OMe, or OMe and OH respectively.
- 13. A compound according to claim 12, wherein  $\mathrm{R'}_2$  is 0,  $\mathrm{CH}_2$  or  $\mathrm{CHCH}_3$ .

- 14. A compound according to either claim 12 or claim 13, wherein  $R_6$ ,  $R_7$  and  $R_9$  and, unless the compound is a dimer,  $R_8$  are independently selected from  $H_7$  OR or a halogen atom.
- 15. A compound according to claim 14, wherein  $R_6$ ,  $R_7$  and  $R_9$  and, unless the compound is a dimer,  $R_8$  are independently selected from H, OMe and OCH<sub>2</sub>Ph, and I.
- 16. A compound according to claim 14, wherein  $R_7$  and, unless the compound is a dimer,  $R_8$  are independently OR or a halogen atom and  $R_6$  and  $R_9$  are H.
- 17. A compound according to claim 16, wherein  $R_7$  and, unless the compound is a dimer,  $R_8$  are independently selected from OMe, OCH<sub>2</sub>Ph or I.
  - 18. A compound according to any one of claims 12 to 17 which is a dimer, wherein the dimer bridge is of the formula  $-0-(CH_2)_p-0-$ , where p is from 1 to 12.

19. A compound of the formula III:

$$\begin{array}{c|c} R_{\theta} \\ \hline R_{1} \\ \hline R_{2} \\ \hline \end{array} \begin{array}{c} N \\ \hline \end{array}$$

wherein:

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 $R_6$ ,  $R_7$  and  $R_9$  are independently selected from H, R, OH, OR, halo, amino, NHR, nitro,  $Me_3Sn$ ;

where R is a lower alkyl group having 1 to 10 carbon atoms, or an aralkyl group of up to 12 carbon atoms, whereof the alkyl group optionally contains one or more carbon-carbon double or triple bonds, which may form part of a conjugated system, or an aryl group of up to 12 carbon atoms; and is optionally substituted by one or more halo, hydroxy, amino, or nitro groups, and optionally containing one or more hetero atoms which may from part of, or be, a functional group;

and  $R_8$  is selected from H, R, OH, OR, halo, amino, NHR, nitro,  $Me_3Sn$ , where R is as defined above or the compound is a dimer with each monomer being the same or different and being of formula III, where the  $R_8$  groups of the monomers form together a bridge having the formula -X-R'-X- linking the monomers, where R' is an alkylene chain containing from 3 to 12 carbon atoms, which chain may be interrupted by one or more hetero-atoms and/or aromatic rings and may contain one or more carbon-carbon double or triple bonds, and each X is independently selected from O, S, or N;

wherein at least one of  $R_6$ ,  $R_7$ ,  $R_8$  and  $R_9$  are not H; except that:

- (i) when  $R_6$  and  $R_9$  are H,  $R_7$  and  $R_8$  are not both OMe, OMe and OBn respectively, or OMe and OH respectively;
- (ii) when  $R_6$  and  $R_7$  are H,  $R_8$  and  $R_9$  are not Me and OH respectively;
- (iii) when three of  $R_6$ ,  $R_7$ ,  $R_8$  and  $R_9$  are H, the other is not Me;
  - (iv) when  $R_6$ ,  $R_7$ , and  $R_8$  are H,  $R_9$  is not OMe;
  - (v) when  $R_6$ ,  $R_8$  and  $R_9$  are H,  $R_7$  is not OMe; and
- (vi) when  $R_6$ , and  $R_9$  are H and  $R_7$  is OMe, the compound is not a dimer.
- 20. A compound according to claim 19, wherein only one of  $R_{6},\ R_{7},$   $R_{8}$  and  $R_{9}$  is H.
- 21. A compound according to claim 20, wherein those of  $R_6$ ,  $R_7$ ,  $R_9$  and, unless the compound is a dimer,  $R_8$  which are not H are OR.
- 22. A compound according to claim 21, wherein those of  $R_6$ ,  $R_7$ ,  $R_9$  and, unless the compound is a dimer,  $R_8$  which are not H are selected from OMe, and OBn.
  - 23. A compound according to either claim 19 or claim 20, wherein at least one of  $R_6$ ,  $R_7$ ,  $R_8$  and  $R_9$  is a dimer, is  $NH_2$ .
  - 24. A compound according to claim 19, claim 20 or claim 23, wherein at least one of  $R_6$ ,  $R_7$ ,  $R_8$  and  $R_9$  is an aryl group, preferably of up to 12 carbon atoms, which is optionally substituted

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by one or more halo, hydroxy, amino, or nitro groups, and optionally contains one or more hetero atoms which may from part of, or be, a functional group.

- 5 25. A compound according to claim 24, wherein at least one of  $R_6$ ,  $R_7$ ,  $R_8$  and  $R_9$ , is a phenyl group, optionally substituted by one or more methoxy, ethoxy or nitro groups.
- 26. A compound according to claim 25, wherein at least one of  $R_6$ , 10  $R_7$ ,  $R_8$  and  $R_9$ , is selected from: Ph, p-MeO-Ph, m-NO<sub>2</sub>-Ph and p-NO<sub>2</sub>-Ph.
  - 27. A compound according to any one of claims 19 to 26 where the compound is a dimer, wherein the dimer bridge is of the formula  $-0-(CH_2)_p-0-$ , where p is from 1 to 12.

#### 28. A compound of formula IV:

$$R^{"_{\delta}} \stackrel{\tilde{N}}{\longrightarrow} 0 \qquad R_{7} \qquad (IV)$$

wherein:

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 $R_6$ ,  $R_7$  and  $R_9$  are independently selected from H, R, OH, OR, halo, amino, NHR, nitro, Me<sub>3</sub>Sn;

- where R is a lower alkyl group having 1 to 10 carbon atoms, or an aralkyl group of up to 12 carbon atoms, whereof the alkyl group optionally contains one or more carbon-carbon double or triple bonds, which may form part of a conjugated system, or an aryl group of up to 12 carbon atoms; and is optionally substituted by one or more halo, hydroxy, amino, or nitro groups, and optionally containing one or more hetero atoms which may form part of, or be, a functional group;
  - $\rm R_8{}^{\prime}$  and  $\rm R_8{}^{\prime\prime}$  are either independently selected from H, R or together form a cyclic amine; and
- 30 n is from 1 to 7.

- 29. A compound according to claim 28, wherein  $R_7$  is an electron withdrawing group.
- 30. A compound according to either claim 28 or claim 29, wherein  $R_6$  and  $R_9$  are selected from H and OR.
- 31. A compound according to claim 30, wherein  $R_6$  and  $R_9$  are selected from OMe, OEt and OBn.
- 32. A compound according to any one of claims 28 to 31, wherein n is 1 to 3.
- 33. A compound according to any one of the preceding claims wherein R is selected from a lower alkyl group having 1 to 10 carbon atoms, or an aralkyl group of up to 12 carbon atoms, or an aryl group of up to 12 carbon atoms, optionally substituted by one or more halo, hydroxy, amino, or nitro groups.
- 34. A compound according to claim 33, wherein R is selected from a lower alkyl group having 1 to 10 carbon atoms optionally substituted by one or more halo, hydroxy, amino, or nitro groups.
  - 35. A compound according to claim 34, wherein R is an unsubstituted straight or branched chain alkyl having 1 to 10 carbon atoms.
  - 36. The use of a compound according to any one of the preceding claims in a method of therapy.
- 30 37. A pharmaceutical composition comprising a compound according to any one of claims 1 to 35 and a pharmaceutically acceptable carrier or diluent.
- 38. The use of a compound according to any one of claims 1 to 35 to prepare a medicament for the treatment of a gene-based disease.

- 39. The use of a compound according to any one of claims 1 to 35 to prepare a medicament for the treatment of a viral, parasitic or bacterial infection.
- 5 40. A process for preparing a compound according to any one of claims 1 to 35.

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- 41. The use of a compound according to any one of claims 1 to 35 for the preparation of a medicament for the treatment of cisplatin-refactory disease.
- 42. A method of inhibiting the growth of cisplatin-refactory cells which method comprises treating said cells with a compound according to any one of claims 1 to 35.
- 43. A method according to claim 42 wherein said compound is SJG-136 1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11aS)-7-methoxy-2-methylidene-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one].

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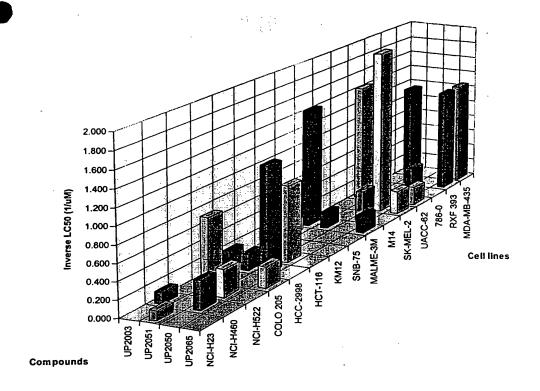
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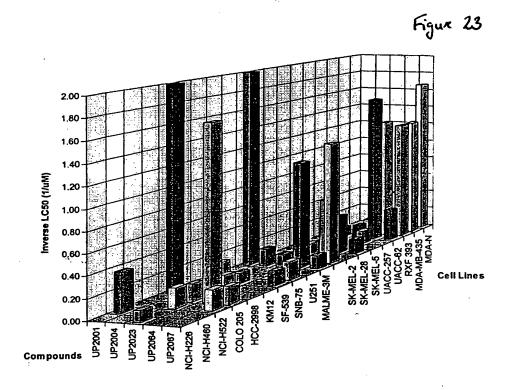
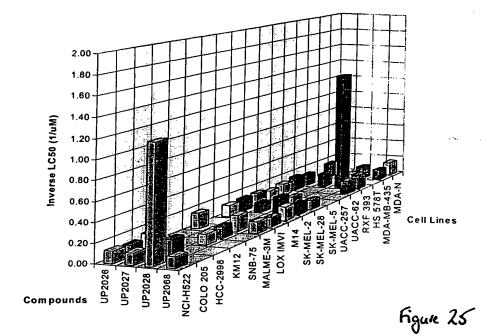
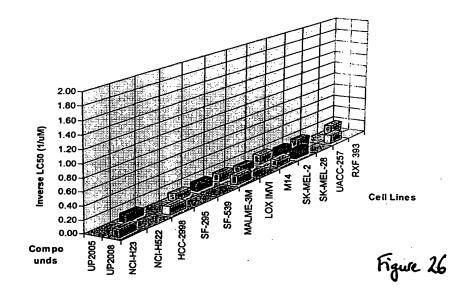


Figure 24

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